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THE DEVELOPMENT AND APPLICATION OF STABLE OXYGEN AND HYDROGEN ISOTOPE ANALYSES OF CHIRONOMIDAE (DIPTERA) AS INDICATORS OF PAST ENVIRONMENTAL CHANGE

A

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Fairbanks, Alaska

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THE DEVELOPMENT AND APPLICATION OF STABLE OXYGEN AND HYDROGEN ISOTOPE ANALYSES OF CHIRONOMIDAE (DIPTERA) AS INDICATORS OF PAST ENVIRONMENTAL CHANGE

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Abstract

Environmental change continues to be of increasing interest to scientists in all disciplines, and there is a paramount need to gain a comprehensive understanding of the impacts of environmental change in the past to better predict the future. A challenge associated with interpreting past change is a lack of reliable proxies to infer past environmental conditions preserved in the fossil record. My research has been dedicated to developing techniques associated with analyzing the stable oxygen and hydrogen isotopic composition ($\delta^{18}O$ and δD) of subfossil chironomid (Chironomidea: Diptera) head capsules (primarily composed of chitin) preserved in lake sediments as a new proxy of past hydrological environmental changes. My developments have included: 1) assessing and modeling the potential of contamination sources during sample preparation; and 2) culturing chironomid larvae under controlled, replicated laboratory conditions, to examine the degree to which water and diet influence the $\delta^{18}O$ and δD of chironomids. My growth experiment demonstrated that 69.0±0.4 % of oxygen and 30.8±2.6 % of hydrogen in chironomid larvae are derived from habitat water using a two-end member mixing model. The $\delta^{18}O$ of chironomids remains can better constrain past habitat water isotopic changes compared to δD, due to 69 % of the chironomid oxygen being influenced by habitat water. Having examined these methodological issues I then applied stable oxygen isotope analyses of fossil chironomid remains preserved in a sediment core from Idavain Lake, in southwest Alaska. The core represents the last ~16,000 years to the present and isotope analyses of chironomids from

the core showed that the $\delta^{18}O$ of past lake water had changed since deglaciation. Large variation in $\delta^{18}O$ of chironomids (up to 20 ‰) are interpreted as alternating shifts in atmospheric flow regimes that are predominant in southwest Alaska, which are consistent with other evidence of past environmental changes at Idavain Lake (i.e. pollen, $\delta^{13}C$, $\delta^{15}N$, C/N). A zonal flow regime appears to have been dominant from 16,000 to 13,800 cal yr BP, 11,000 to 10,500 cal yr BP, 10,000 to 8,000 cal yr BP, and during a majority of the periods from 8,200 to 3,500 cal yr BP and from 2,000 cal yr BP to present. A mixed modern flow regime seems to have been dominant during the periods from 13,000 to 11,000 cal yr BP, 10,500 to 10,000 cal yr BP, 6,000 to 5,500 cal yr BP and 2,500 to 1,800 cal yr BP. These shifts in moisture regime appear to coincide with a series of glacier advances and recessions along the Gulf of Alaska. Thus, stable isotopic analysis of chironomid headcapsules is a promising tool for indicating paleoenvironmental change.

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List of Abbreviations

ASIF – Alaska Stable Isotope Facility

EA – Elemental Analyzer

H_2 – Hydrogen gas

HCl – Hydrochloric Acid

IRMS – Isotope Ratio Mass Spectrometer

KOH – Potassium Hydroxide

TC/EA – Temperature Conversion Elemental Analyzer

VSMOW – Vienna Standard Mean Ocean Water

VPDB – Vienna Pee Dee Belemnite

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CHAPTER 1

General introduction

Background

The Subarctic and Arctic region are highly sensitive to climate changes (PARCS, 1999). Changes occurring at higher latitudes will likely influence the global climatic system. The likelihood of dramatic environmental changes present a growing interest and concern to scientists in all disciplines, and there is now a paramount need to gain a more comprehensive understanding of the impacts of climate change on the past to better predict possible changes in the future. However, the short time span of direct observations limits our ability to evaluate the roles of natural climate variability in explaining the warming trends. The range of natural climate variability in the Subarctic and Arctic can be evaluated by reconstructing past environmental conditions since the last deglaciation, which requires reliable proxies of past environmental conditions and continuous archives of these proxies at suitable temporal resolution. In the terrestrial realm, lake sediments are particularly useful archives of paleoclimate information because they can provide continuous records and contain multiple geochemical and microfossil proxies that can be used to provide paleoclimate signals (Battarbee, 2000; Birks, 1998; Meyers, 1994; Smol, 2002).

One approach to decipher past environmental conditions is to reconstruct the past oxygen and hydrogen stable isotopic compositions of precipitation. Changes in storm-

track trajectories and moisture origins can result in substantial geographic gradients in the stable isotopic compositions ($\delta^{18}O$ and $\delta^{2}H$) of precipitation (Dansgaard, 1964; Gat, 1996; Gat, 2000; Gat, 2008; Rozanski et al., 1993), and therefore, local water (Bowen et al., 2005; Ehleringer et al., 2008; Hobson, 2005; Hobson et al., 1999; O'Brien and Wooller, 2007). Organisms can record the isotopic composition of these source waters in their organic compounds. In plants, for instance, the stable isotopic composition of oxygen in cellulose, including cellulose preserved in lake sediments, is correlated with that of source water and has been used to indicate past temperatures and effective moisture (Anderson et al., 2001; Sauer et al., 2001). Stable oxygen isotope analyses have also been conducted on carbonate precipitated on *Chara* sp. (Anderson et al., 2001) and ostracod shells (Hu et al., 2003) to reconstruct past climate. However, the use of materials composed of carbonate (e.g. ostracod shells) is somewhat limited because carbonate does not preserve in lakes with low pH (Hu et al., 2003). Meanwhile, lake sediment derived paleo-isotopic studies are frequently hampered by difficulties in isolating a purely autochthonous sedimentary material. For instance, sedimentary cellulose can contain large proportions of terrigenous matter and failing to separate the aquatic cellulose source from a terrestrial cellulose source can complicate paleoenvironmental reconstructions (Sauer et al., 2001; Wolfe et al., 2000; Wolfe et al., 2007). Stable isotope analyses of diatoms (biogenic silica) is also challenging due to the difficulties of separating non-biogenic and biogenic silica (Lamb et al., 2004; Lamb et al., 2005; Leng and Barker, 2006).

Oxygen stable isotopic analyses of chironomid chitinous remains preserved in lake sediments have been used in paleoecological studies to infer the past isotopic compositions of lake water (Wooller et al., 2004; Wooller et al., 2007). This approach has good potential because chironomids spend their entire larval stages (i.e. instars) living in water and the chitin (chironomid head capsules) from these stages remain preserved in lake sediments (Walker, 1995; Walker, 1987; Walker, 2001). The family Chironomidae is also widely distributed and are abundant in most aquatic habitats, even those in extremely cold environments such as those in Antarctica (Gullan and Cranston, 2000; Oliver, 1971). Changes in chironomid faunal assemblages over time have been used to infer past environmental change (i.e. lake water temperature, salinity and oxygen availability) based on modern calibration data and transfer functions (Brodersen et al., 2004; Clerk et al., 2000; Heinrichs et al., 1999; Langdon et al., 2004; Langdon et al., 2006). Stable isotope analyses on chironomid chitin can be a complimentary addition to paleolimnological research using chironomid head capsules. Although oxygen isotope analyses of chironomids have shown promise for reconstructing past environmental conditions (Wang et al., 2008; Wooller et al., 2004; Wooller et al., 2007), there have been few applications of this approach to date. One limitation on applying this approach is that the methods have not been explicitly described and evaluated. Also the degree to which both the isotopic composition of lake water and diet influence the stable isotopic composition of chironomids has not been explored. The goals of my PhD study are to fill these knowledge gaps.

Scope of the study

The objectives of this research were threefold: 1) Evaluate and optimize the method for analyzing the isotopic composition of chironomid head capsules; 2) Evaluate the degree to which water and diet influence the stable isotopic composition (oxygen and hydrogen) of chironomids. The results from this study will allow more critical interpretation of paleo data; 3) Analyze the stable isotopic composition of chironomid remains preserved in a lake core from southwest Alaska and use these data to reconstruct past environmental conditions. With these goals in mind, a description of the following chapters is presented below.

Chapter 2 presents results of a series of methodological considerations related to analyzing small organic samples for their oxygen and hydrogen stable isotopic composition using a continuous flow temperature conversion elemental analyzer (TC/EA) attached to an isotope ratio mass spectrometer. The potential for a blank effect associated with silver and tin capsules that are typically used to contain samples for analysis was evaluated. The effects of blanks of varying sizes and isotope compositions on sample analyses were also modeled, to provide guidelines for minimum sample sizes and to assess the degree of blank tolerance. A protocol for preparing chironomids for stable isotope analyses is subsequently outlined. The content of this chapter was published in the *Journal of Paleolimnology* in March 2008 (Wang et al., 2008).

Chapter 3 examines the influence of water and diet oxygen and hydrogen isotopic compositions on the oxygen and hydrogen isotopic composition of chironomids using a

laboratory-based growth experiment. Two isotopically different (oxygen and hydrogen) growth waters were used in aquaria containing chironomid eggs masses. These eggs were allowed to develop into larvae. The oxygen and hydrogen stable isotopic compositions of chironomids, their food, and aquaria water were then used to calculate the proportional contributions of oxygen and hydrogen from water vs. diet to the organic composition of chironomid larvae. These data are needed because it is important to know to what degree chironomids reflect the water they live in, which will aid evaluations of paleo data based on isotope analyses of chironomid remains.

Chapter 4 presents an application of the stable isotope techniques described in Chapter 2. The stable oxygen and hydrogen isotope composition of chironomid remains (head capsules) preserved in a core of sediment taken from Idavain Lake, Southwest Alaska are presented in concert with a suite of other paleolimnological analyses (i.e. loss of ignition, carbon and nitrogen ratio, and magnetic susceptibility) of the core to reconstruct paleoenvironmental changes over the last ~16,000 years.

Chapter 5 reports the general conclusions of the results of the previous chapters, and summarizes the potential of stable isotopes analyses of chironomids in paleoecological research. General impacts of this study on future paleoclimatological and paleoenvironmental changes are included in closure.

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CHAPTER 2

A protocol for preparing subfossil Chironomid head capsules (Diptera: Chironomidae) for stable isotope analysis in paleoclimate reconstruction and considerations of contamination sources[1]

Abstract

Several techniques are available to examine the isotopic composition of historic lake waters, providing data that can subsequently be used to examine environmental changes. A recently-developed technique is the stable oxygen isotope analysis of subfossil chironomid (Diptera: Chironomidae) head capsules (mostly chitin) preserved in lake sediments. This technique involves a high Temperature Conversion Elemental Analyzer (TC/EA), which has been a relatively recent addition to the suite of online peripherals for analyzing the stable isotopic composition of organic samples. The highly precise and accurate $^{18}O/^{16}O$ and D/H measurements obtainable using the TC/EA with samples in the microgram range make this instrumentation suitable for studying geochemical and biological processes. Preparation of organic samples for isotopic analysis typically requires first weighing each sample into silver/tin capsules. These capsules can introduce oxygen and hydrogen contamination (a "blank effect"), which is especially problematic

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[1]Wang, Y., O'Brien, D., Francis, D., Wooller, M.J. 2008. A protocol for preparing subfossil Chironomid head capsules (Diptera: Chironomidae) for stable isotope analysis in paleoclimate reconstruction and considerations of contamination sources. Journal of Paleolimnology. DOI 10.1007/s10933-008-9197-3

for analysis of small organic samples (e.g. less than 100μg). Here we tested tin and silver capsules from two manufacturers and a range of sizes to assess contamination to small organic samples on the TC/EA. We also assessed how a method for cleaning silver capsules affected our analysis of commercial chitin. In general, capsules made of silver have less detectible oxygen than those made of tin, and capsules from the two manufacturers varied in their detectible oxygen. There was no detectable H contamination from silver capsules. In addition to our empirical findings, we present a model demonstrating the influence that contaminant oxygen can have on the $\delta^{18}O$ of small organic samples. Sample mass becomes an important issue for such analyses. In light of our findings, we recommend a minimum sample mass $\geq$ 50μg (approximately 120 whole chironomid head capsules) on a TC/EA-IRMS (Delta^{plus} XP system). Finally, we present a detailed protocol for preparing and transferring chironomid head capsules into silver capsules that minimizes the influence of contaminant oxygen. This protocol provides the paleo-community with another potential method for reconstructing paleoenvironments.

Introduction

Analyses of the stable oxygen and hydrogen isotope composition of biogenic materials have been used to reconstruct the stable isotopic composition of past precipitation, which is subsequently used to infer past environmental changes. The most commonly used methods in limnological isotope studies are the $\delta^{18}O$ analyses of carbonates from benthic ostracods (Hu et al., 2003), *Chara* sp. calcites (Anderson et al., 2001), biogenic silica (Lamb et al., 2004; Lamb et al., 2005; Leng and Barker, 2006) and aquatic cellulose (Anderson et al., 2001; Sauer et al., 2001; Wolfe et al., 2000; Wolfe et al., 2001; Wolfe et al., 2007). The δD of beetle chitin (Grocke et al., 2006), aquatic biomakers (Huang et al., 2004) and total organic matter (Wooller et al., 2007) have also been used to examine the past isotopic composition of precipitation and paleoclimate change.

These analyses have been facilitated by the development of online continuous flow instruments for stable isotope analysis of organic and inorganic samples, in particular, the High Temperature Conversion Elemental Analyzer (TC/EA) (Farquhar et al., 1997; Kelly et al., 1998; Kornexl et al., 1999a; Koziet, 1997; Werner, 2003; Werner et al., 1996), which determines D/H and $^{18}O/^{16}O$ stable isotope ratios in a rapid, easy, and precise fashion (Koziet, 1997; Sharp et al., 2001). This on-line practice has reduced the sample mass required for analysis by an order of magnitude (Sharp et al., 2001; Werner et al., 1996), allowing analysis of samples in the microgram range (Farquhar et al., 1997; Kornexl et al., 1999a) and has made the technique suitable for studying natural geochemical and biological processes.

$\delta^{18}O$ analyses of subfossil chironomid (Diptera: Chironomidae) head capsules in lake sediments via TC/EA have been proposed as a way of examining past environmental conditions (Wooller et al., 2004; Wooller et al., 2007). Despite the potential of this approach, an explicit description of a protocol for the preparation of chironomid head capsules (hereafter referred to as fossil heads) for stable isotope analyses (C, N, H and O) using the TC/EA is lacking from the current literature. The paper by Wooller et al. (2004) described a modern calibration set and two examples of the method's application to paleo reconstruction, but did not present the steps involved in preparing the samples. Recent studies devoted to $\delta^{18}O$ analyses of biogenic materials (e.g. cellulose, diatoms and human hair) have emphasized the need for careful attention to the details associated with sample-handling protocols (Bowen et al., 2005a; Leng and Barker, 2006; Wassenaar and Hobson, 2002; Wolfe et al., 2007). Here we contribute to this literature with a detailed assessment of methodological considerations for analyzing the $\delta^{18}O$ and δD of fossil chironomid heads via TC/EA.

Previous work with fossil chironomid heads required very large numbers (300-700) to obtain enough sample mass for analysis (Wooller et al., 2004), and was time consuming. Reducing the sample mass (fossil head numbers) can result in a decrease in the sample voltage signal to noise ratio during $\delta^{18}O$ analyses. This occurs if empty tin or silver capsules (vessels used to contain samples for analysis) are contaminated with oxygen and hydrogen. The result is an oxygen or hydrogen voltage signal from the empty, or blank, capsules (hereafter referred to as a 'blank' effect) (Farquhar et al., 1997; Werner, 2003; Werner and Brand, 2001; Werner et al., 1996). The influence of the blank

effect is naturally much greater when small sample masses are analyzed. A 'blank' signature has the potential to significantly affect the accuracy and precision of analyses of small-sized organic samples.

Capsules used for TC/EA analysis of organic samples are available in both silver and tin, and come in a variety of sizes. One might hypothesize that capsule sizes may influence the blank differently, for instance, the larger capsules may carry a larger blank signature. The different capsule material (either tin or silver) could also influence the blank differently. However, analyses in the published literature using the TC/EA have been conducted using both tin and silver capsules (Farquhar et al., 1997; Kelly et al., 1998; Kornexl et al., 1999a; Kornexl et al., 1999b; Kreuzer-Martin et al., 2003), and a direct comparison of their blank effects has not been conducted. Tin capsules are cheaper, leave fewer residues in the reactor furnace of the TC/EA (Alaska Stable Isotope Facility, personal communications), and don't react to acidic samples (detailed discussions see ISOGEOCHEM item number: 014870). However, Thermo Finnigan INC. suggests using silver capsules (Thermo Finnigan operation manual, 2003) for isotopic analysis on the TC/EA connected to an Isotope Ratio Mass Spectrometer (IRMS), because the glassy carbon reactors can be easily cleaned after running silver capsules whereas the whitish-grey coat left behind after using tin capsules are not so easily removed (SIRFER laboratory, personal communication; ISOGEOCHEM Listserv archive item number: 014856). Different users weigh these advantages and disadvantages when deciding which type of capsule to use, and as yet there is no clear consensus. Here we investigate the methodological considerations behind the analysis of small organic

samples for $\delta^{18}O$ (and δD) using continuous flow TC/EA isotope ratio mass spectrometry. We examined the blank effect associated with silver and tin capsules of a variety of sizes from multiple batches and two different manufacturers. We also tested whether a cleaning procedure will decrease the blank effect. In addition, since chironomid sorting and transferring involves distilled water as a medium, we also tested whether wetting silver/tin capsules influenced the blank signal. We also modeled the effect of blanks of varying size and isotope composition on sample analyses of $\delta^{18}O$, to provide guidelines for minimum sample sizes and blank tolerance. Finally, we provide a clear outline of a protocol for preparing chironomids for stable isotope analysis to promote this new proxy for studying environmental changes.

Materials and methods

Assessment of blank effects from empty capsules

We measured the blank effect associated with different sizes of silver and tin capsules from two manufacturers (Manufacturer 1: silver: 5x3.5mm, 6x4mm, and 9x5mm; tin: 5x3.5mm and 6x4mm; and Manufacturer 2: silver: 4x3.2mm, 3.75x3.5mm, and 6x4mm; tin: 3.75x3.5mm and 4.74x4mm) (Table 2.1). The capsules are packaged in plastic jars by the manufacturers, and batch numbers are provided to denote the production at different times. Therefore, several jars and batches were chosen to examine the degree of variability on blank signatures. The capsules to be analyzed were folded into balls and

stored in 96-position culture trays (Elisa plates) covered with a lid. The capsules were then loaded into a zero blank auto sampler attached to an on-line pyrolysis thermochemical reactor (ThermoElectron TC/EA) coupled via a Conflo III with a thermoFinnigan Delta^{plus} XP IRMS at the Alaska Stable Isotope Facility (ASIF). In order to examine any wetting/drying effect on the empty silver capsules, we also rinsed a series of different sized capsules (silver and tin) from manufacturer 1 and all sizes of capsules except for size 6x4mm silver and 4.75x4mm tin capsules from manufacturer 2 with Type I ultrapure water (18.2 MW·cm resistivity at 25 °C and < 10 ppb Total Organic Carbon). These capsules were left to thoroughly air dry for 24 hours and were then analyzed as above. Blank signals were reported as voltage peaks generated by the oxygen and hydrogen mass from blank capsules on the Delta^{plus} XP IMRS. The minimum peak detection limit for the TC/EA used at ASIF is smaller than 50mV (0.05V).

Examination of a cleaning treatment

Some laboratories and manufacturers clean silver capsules before loading samples to reduce the blank effect. We examined the effect of applying a cleaning method for silver capsules on $\delta^{18}O$ and δD analyses of a chitin standard. Silver capsules (size 5x3.5mm) from manufacturer 1 were selected for examining the cleaning method because this type produced undetectable oxygen in initial tests.

Thirty 5x3.5mm silver capsules were rinsed twice using 100% acetone and left to air-dry for 24 hours. Thirty-seven untreated 5x3.5mm silver capsules were used as a

control. We used pure homogenous chitin (made of crab shell, Sigma Organic Co. 2002) as our sample material. Chitin was weighed into the capsules using a microbalance (M2P Sartorius, repeatability 0.001mg). Sample mass varied between 0.016 to 0.427 mg. All of the capsules were then folded into a small ball and analyzed on the TC/EA connected with IRMS at the ASIF. Standards (NBS18, NBS19, NBS22, NBS30, ANU sucrose, IAEA-601 and IAEA-602), and an internal organic (keratin) laboratory standard (BWBII) were analyzed with each run of chitin samples (measured vs. expected $r^2 > 0.98$). ASIF internal laboratory standard benzoic acids (Fisher Scientific, No. 947429) were analyzed after every ten samples as a precision check. Analytical precision for $\delta^{18}O$ and δD is 0.6‰ and 4‰, respectively. All chitin results of $\delta^{18}O$ and δD are reported in units of per mil (‰) relative to Vienna Standard Mean Ocean Water (V-SMOW): $\delta X = (R_{sample}/R_{standard} - 1) \times 1000‰$, where X is oxygen, R is the ratio of heavy to light isotope, and the standard is Vienna Standard Mean Ocean Water (V-SMOW). A one-way ANOVA was performed to compare chitin $\delta^{18}O$ using the cleaning methods against the control. We also compared the variances of $\delta^{18}O$ from the cleaning treatment and the control using Brown-Forsythe's test. All analyses were performed using the statistical package SAS 8.0.

Modeled influence of blanks on stable oxygen isotope analyses

A two end-member mixing model [1] was used to illustrate the contribution of isotopic composition from sample and blank sources:

$$\delta^{18}O_M = \delta^{18}O_S * p_S + \delta^{18}O_B * p_B \qquad [1]$$

$$1 = p_S + p_B \qquad [2]$$

Where M = Measured, S = Sample, B = capsule Blank effect, and p_S and p_B = the oxygen proportional contribution of sample and blank, respectively. This expression has 3 unknowns: p_S, $\delta^{18}O_S$, and $\delta^{18}O_B$, and is the same for δD measurements. By including several blank capsules in the sample sequences, the $\delta^{18}O_B$ and the magnitude of the blank effect (in volts) can be measured. The p_B can be calculated for each sample as p_B = Blank peak area / Total peak area. The isotopic composition of individual samples ($\delta^{18}O_S$) can therefore be corrected by rearranging the mixing model [1] and [2].

$$\delta^{18}O_S = \frac{\delta^{18}O_M - \delta^{18}O_B \times p_B}{1 - p_B} \qquad [3]$$

Correction for a blank effect is usually small when a large sample mass is used. When analyzing small-mass samples, however, the correction can be significant. Here we varied $\delta^{18}O_B$ and p_B to explore the effect of blanks of differing size and isotopic composition on $\delta^{18}O_M$. We used two model scenarios. In the first model [1] we kept $\delta^{18}O_S$ and the blank oxygen proportional contribution constant (e.g. $p_B = 0.6$), and varied the isotope signature of the blank ($\delta^{18}O_B$) from -20 to 10 ‰ (these values are within the range we detected $\delta^{18}O_B$ in our experiments with blanks at ASIF). We assigned the value

of $\delta^{18}O_S$ to be 20.5‰, the mean of the standard chitin measured in ASIF (see above). We then performed this simulation over a range of other possible blank oxygen percentage contributions (p_B =0, 0.15, and 0.3). These ranges of blank contributions were selected in the model simulation because we have experienced blank oxygen proportional contributions as high as 0.6 in prior analyses with small sample masses. In the second model we varied the percentages of the blank oxygen contribution but kept $\delta^{18}O_B$ (-7.7‰, the mean blank $\delta^{18}O_S$ value from our experiments) and $\delta^{18}O_S$ (20.5‰, as a mean value for chitin analyzed at ASIF) constant.

A protocol for preparing chironomids for stable isotope analysis

We investigated methods for cleaning and transferring chironomid fossil heads from sediment samples into silver capsules for stable isotope analysis. Here we present a description of the preparation protocol that we determined to be the most efficient (Fig. 2.1). About two to four cubic centimeters (cc) of lake core sediment is often sufficient to obtain enough chironomid fossil heads. Extraction of fossil heads from sediments follows the procedure described by Walker (2001). Samples are first treated with 10% HCl for 24 hours, and then 5% KOH in a warm (60-70°C) water bath for 15-20 minutes. Between HCl and KOH treatment, the sediments are rinsed with distilled water in a 100μm sieve (Walker, 2001). After these processing steps, the residual material is refrigerated in a 50ml centrifuge tube until it is ready for sorting.

The treated samples are sorted with two hand picking and transfer steps (Fig. 2.1). In the first transfer step, small aliquots of aqueous residual material are transferred using a pipette into a Bogorov counting tray (Gannon, 1971) and examined under a dissecting microscope at 25-50X magnification. Fossil chironomid heads are transferred using fine forceps into 5ml plastic centrifuge vials (VWR Scientific Product) containing Type I ultrapure water (hereafter, "water"). The vials are stored at 4°C until ready to proceed with the next transfer step. The second step involves transferring the fossil heads into a silver capsule for isotopic analysis. Excess water is removed from the centrifuge tube carefully so as not to disturb fossil heads that have settled to the base of the tube from the first step. The remaining water and concentrated heads are then gently poured into a concave watch glass. We use a few extra drops of water to rinse the tube and ensure that no chironomid fossil heads remain stuck to the inner wall of the centrifuge tube. Using fine forceps and a dissection scope at 50X magnification, a minimum of 120 chironomid heads are transferred into pre-weighed silver capsules filled with water. If water evaporates during the process, additional water sometimes is added to refill the capsules. In order to prevent the silver capsules from tipping over, an inexpensive holder can be made from an appropriately sized nut from a nut-and-bolt assembly. When all the heads are transferred into the silver capsules, the open silver capsules are allowed to air dry over night. The capsules are then crimped carefully to leave a small opening and freeze dried for ≥ 6 days (Bowen et al., 2005). The silver capsules are then reweighed and the mass of each sample is calculated by subtracting the tare weight. Silver capsules are then folded into a tiny silver ball and stored in an Elisa culture tray in a freeze drier or a

vacuum system prior to loading them into a zero blank auto-sampler attached to the TC/EA-IRMS described above for the stable isotope analysis. Benzoic acid and blank silver capsules are placed into the auto-sampler along with other international standards as described in the chitin experiments above.

It is worth noting that during sedimentation processes and chemical treatments, some mouthparts may be lost or detached or whole heads can break into two pieces. We experienced that many fossil heads tend to break along the middle line, so every two half heads were counted as one.

Results

Assessment of blank effects from empty capsules

The blank effects (in volts) from silver and tin capsules over a range of sizes and from two manufacturers are presented in Table 2.2. All sizes (5x3.5mm, 6x4mm, and 9x5mm) of silver capsules from manufacturer 1 showed no detectable oxygen blank signal (Table 2.2 and Fig. 2.2), either unrinsed or rinsed. Only size 9x5mm silver capsules produced a detectable hydrogen blank signal, and did so for both rinsed and unrinsed capsules (mean = 0.02 (±0.01) volts). Both sizes of tin capsules from manufacturer 1 had detectable oxygen blank voltages, and a blank hydrogen voltage was observed in the 6x4mm tin capsule only. Rinsing appeared to cause all blank voltages, when present, to increase slightly. Although production batch numbers were not supplied by manufacturer 1, the

5x3.5mm silver capsules were ordered in both 2003 and 2004, which both produced undetectable blanks. One volt of oxygen is generated by ~30μg oxygen sample mass on the Delta^{plus} XP TC/EA-IRMS system at ASIF. One volt of hydrogen is generated by ~5.5μg hydrogen sample mass.

All unrinsed and rinsed silver and tin capsules from manufacturer 2 produced detectable oxygen blank signals, but no detectable hydrogen blank signals (Table 2.2 and Fig. 2.2). Rinsing appeared to decrease the blanks slightly. However, there were no significant differences in oxygen signals from rinsed and unrinsed capsules (all $p>0.06$ for each size). The three sizes of silver capsules (n=55) showed a significant difference in their oxygen blank ($p<0.0001$, ANOVA). The smaller the size of silver capsules from manufacturer 2, the less the oxygen blank effect. Oxygen blanks from two sizes of tin capsules (n=32) did not show significant differences ($p=0.37$). We compared two production batches of each capsule size from manufacturer 2, and found no significant differences in the amount (voltage) of blank (*t*-test, all $p\geq0.14$) except for 3.75x3.5mm silver capsules ($p=0.04$).

The detectable blank $\delta^{18}O$ produced from capsules (n=34) ranged from -31 to 10 ‰ with a mean of -7.7 (±10.9) ‰. The repeatability of these blanks was low.

Examination of a cleaning treatment

The chitin contained in silver capsules (n=42) that had been rinsed in acetone yielded a mean $\delta^{18}O$ of 22.2‰ (±1.3), whereas the chitin from untreated silver capsules (n=37)

yielded a mean of 20.6‰ (±0.7) (Table 2.3 and Fig. 2.3). The variance in the chitin $\delta^{18}O$ from the untreated capsules was significantly lower than acetone rinsed capsules ($p<0.0001$, Brown-Forsythe variance test). Measurements of chitin $\delta^{18}O$ were highly consistent from those untreated capsules, with the exception of samples having voltages below 0.39 V (Fig. 2.2). For small sample masses (< 0.50 volts ≈ 50μg sample) the signal to noise ratio becomes large. If we remove these small voltages (< 0.50V), the mean value of pure chitin in untreated silver capsules (n=33) was 20.5‰ (±0.2) (reported here as the true value of chitin from the untreated silver capsules), whereas acetone rinsed silver capsules (n=36) yielded a mean $\delta^{18}O$ of 21.9‰ (±1.2).

Modeled influence of blanks on stable oxygen isotope analyses

The $\delta^{18}O$ values for small organic samples are composed of two end-members: the organic sample oxygen and the capsule contaminant or blank oxygen. Our model assumes that $\delta^{18}O_S$ (20.5‰) and blank oxygen contribution are constant as 0.60, but $\delta^{18}O_B$ varies from -20 to 10‰ (Fig. 2.4a). The measured sample $\delta^{18}O_M$ is positively correlated with the $\delta^{18}O_B$ and the $\delta^{18}O_M$ have a wide range of values. We repeated the model by assigning different blank oxygen contributions (e.g. 0, 0.15, and 0.30). The smaller the oxygen percentage from the blank capsules, the closer the measured $\delta^{18}O_M$ to the corrected $\delta^{18}O$ (true sample value). In other words, the slope of correction decreases as the percentage of blank oxygen contribution decreases. When the blank oxygen from the capsules is 0%, the measured $\delta^{18}O$ is the true sample value. In model scenario 2 we

kept the $\delta^{18}O_B$ (-7.7‰, the mean of $\delta^{18}O_B$) and $\delta^{18}O_S$ (20.5‰) constant, and varied the ratio of sample and blank capsule oxygen contribution. The result (Fig. 2.4b) showed that when sample oxygen percentage is much greater than the blank oxygen percentage (the ratio of sample and blank capsule oxygen contribution is large), the measured value $\delta^{18}O_M$ is closer to the true sample value $\delta^{18}O_S$.

A protocol for preparing chironomids for stable isotope analysis

Using silver capsules with no measurable blank allowed us to decrease the number of chironomid fossil heads from 300-700 chironomid fossil heads to approximately 120 (>50μg) with a variety of sizes, and still produce an adequate signal (at least 0.5 volts on the Delta^{plus} XP TC/EA-IRMS at ASIF). This new approach has been used to analyze stable isotopes (C, N and O) on chironmid fossil heads from a lake core from Northern Iceland (Wooller et al., 2007). Triplicate analyses of chironomid fossil heads (>50μg) from the same depth from a core of lake sediment from an Icelandic lake produced a standard deviation of 0.6‰ (Wooller et al., 2007).

Discussion

Our analyses of the blank effects from empty capsules showed that blank oxygen and hydrogen voltages were dependent on the manufacturer, materials (silver and tin), and capsule size. Type I ultrapure water rinsing did not affect the oxygen blank signals

significantly on any types and sizes of capsules, which ensured that using water as a medium during chironomid transferring and picking has no detectable influence on the blank effect. Our numerical model showed that the $\delta^{18}O$ and percentage of the blank signal from tin or silver capsules can have a significant influence on the $\delta^{18}O$ of small organic samples. Silver capsules from manufacturer 1 had the lowest detectible oxygen voltages compared to tin capsules. This finding is consistent with the discussions on ISOGEOCEM (item number: 014854). Tin capsules of different sizes (5x3.5mm and 6x4mm) from manufacturer 1 showed significant differences in both oxygen and hydrogen blank voltages. The larger the capsule size, the larger the blank signals.

Significant differences in oxygen blank signals between different sizes of silver capsules were shown from manufacturer 2, which may suggest capsule size needs to be taken into consideration when sample sizes are limited. A majority of batches in the same size capsules have no significant differences of blank, although we observed that significant batch differences (3.75x3.5mm silver capsules) of blank effect do occur. We therefore recommend restricting the capsules used in a particular study to one particular batch. We did not see significant differences in the two sizes of tin capsules from manufacturer 2. However, even though there were blank signals detected on the instrument, they were still very small in terms of their voltages compared to the voltage generated by a larger sample (>1000μg). Thus we recommend use of silver capsules when sample sizes are limited. We also recommend correcting for the blank effect by analyzing a series of blank silver or tin capsules used in sample preparation. Because the blank effect varies between manufacturers, we recommend that each stable isotope

laboratory assess several manufacturers for different capsule sizes and materials before analyzing samples. A blank correction procedure comes with TC/EA operating software. However, manually conducted blank correction may be more appropriate because of the uncertainty of blank $\delta^{18}O$ and capsule size in use.

We have also noticed that silver capsules oxidized when stored containing benzoic acid for a considerable time. Oxidization has also been observed when silver capsules were used to contain other acidic materials such as tomato juice (see discussion in Listserv in ISOGEOCHEM: Item No.014857). Therefore, when handling acidic samples, we recommend avoiding silver capsules. We also recommend weighing benzoic acid standards just prior to analysis to avoid oxidization of silver capsules.

Although methods of cleaning silver capsules have been recommended by other laboratories, we found that the cleaning method we investigated were not necessarily effective at removing the contaminants from the silver capsules. We found that pure chitin in silver capsules that had been cleaned via acetone rinse yielded data with greater variance compared to those samples prepared in untreated silver capsules from the same manufacturer. The precision was markedly better for untreated capsules. We have also found (data not shown here) that chitin samples weighed into 5x3.5mm silver capsules that had been baked in a high temperature furnace (400 °C, purged with N_2 gas) for 24 hours also showed large variance (Wang, unpublished data).

Our results showed the minimum chitin size to be ~50μg (0.5 volts) for analysis on our Delta^{plus} XP system TC/EA-IRMS, however, we recommend even larger sample masses (to at least generate 1volt) to further improve precision on this system (Fig. 2.3).

The relatively newer Delta V IRMS also has much higher sensitivity compared to a Delta^{plus} XP. In this case, fewer chironomid heads would be required in order to yield a detectable signal. A test of the minimum oxygen sensitivity is required at the individual laboratory when considering measurement of relatively small samples mass.

Stable isotope analysis of chironomid fossil head capsules is a relatively new technique and only a few published applications have thus far been completed using this proxy. Compared to the first report using this technique (Wooller et al., 2004), this new study has significantly decreased the numbers of chironomid fossil heads (sample mass) needed for $\delta^{18}O$ analysis, which makes this approach less time consuming. We have divided the transferring procedure of chironomid fossil heads for isotope analysis into two steps, compared to Wooller et al. (2004), where the chironomid fossil heads were directly picked from a Bogorov counting tray into a silver capsule. One of the disadvantages of a one step transfer is that the sorting time is long, thus the first sample of one batch can end up stored in silver capsules for an extensive period of time compared to the last sample prepared. In the new protocol, an entire batch of samples can be sorted and stored in ultrapure water before proceeding with the second transfer step. This ensures that all samples in one batch are stored in the silver capsules for about the same length of time. The second transfer step also allows removal of other accidentally picked materials or misidentified parts from the sample. Chironomid fossil heads picked and transferred in this manner can also be used as standard protocol for stable isotope analyses of other elements such as carbon, nitrogen (Wooller et al., 2007) and hydrogen. The smaller sample mass and two step transferring protocol have resulted in much faster

sample processing. However, even though we recommend a minimum of ~120 chironomid fossil heads (equivalent to 0.5 volts of oxygen signal on a Delta^{plus} XP system at ASIF) the number should be based on the minimum (or greater) sample mass and the sensitivity of the instrument that is used. Chironomid heads also range in size due to their different instar stages, or different species. We have experienced some samples in which 50 large fourth instar larvae heads in an Icelandic lake core generated 50μg (equivalent of ~0.5 volts) and produced repeatable data (Wooller et al., 2007). Therefore the procedure for chironomid picking can be shortened if large instar larvae are preserved in the sediments. It is the sample mass that is the essential factor for organic isotopic analysis. We also suggest that other researchers assess the relative abundances of chironomid fossil heads from several depths before dedicating time to downcore $\delta^{18}O$ analyses.

Additional recommendation and future direction

Even though $\delta^{18}O$ of chironomids has been applied as a new proxy for past environmental changes, the complex origin of oxygen in chitin is not fully understood, since both water and diet can influence the $\delta^{18}O$ of chitin (Miller, 1991; Miller et al., 1993; Schimmelmann and DeNiro, 1986). The degree to which water, diet, and physiology (e.g. temperature dependent fractionation) influence $\delta^{18}O$ in chitin is largely unexplored. The fundamental mechanisms controlling $\delta^{18}O$ in chironomids still need to be quantitatively understood. Therefore experimental, laboratory-based validations of isotope effects under a variety of conditions could provide an excellent model for

simulating different environmental parameters to examine their influence on stable isotope composition (Gannes et al., 1997). Understanding the influence of water and diet on tissue $\delta^{18}O$ and the magnitude of temperature dependent physiological isotope fractionation will indeed lay the groundwork for a variety of paleoecological applications using $\delta^{18}O$.

Acknowledgements

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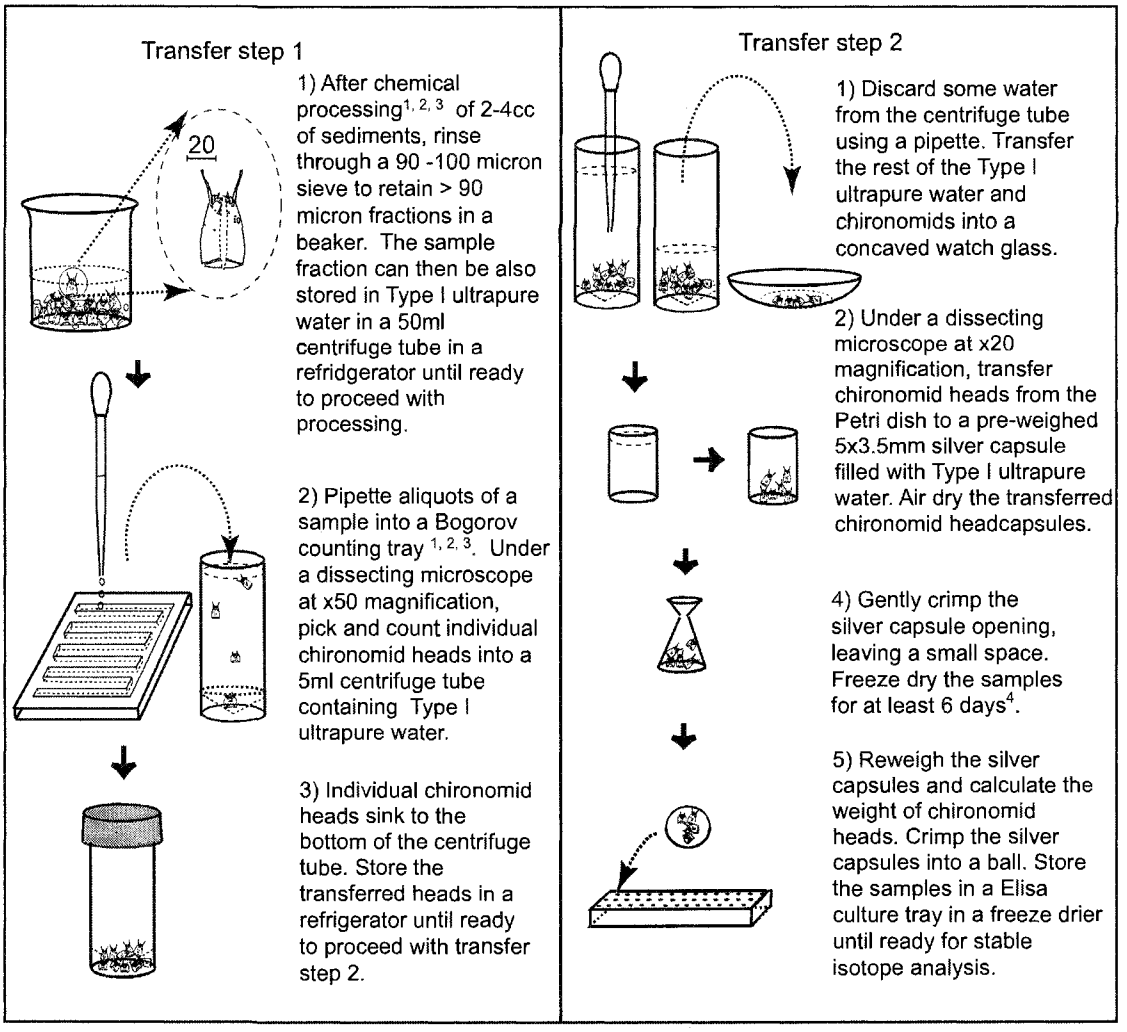

Fig. 2.1 Schematic description of a chironomid transferring protocol for stable isotope analysis.

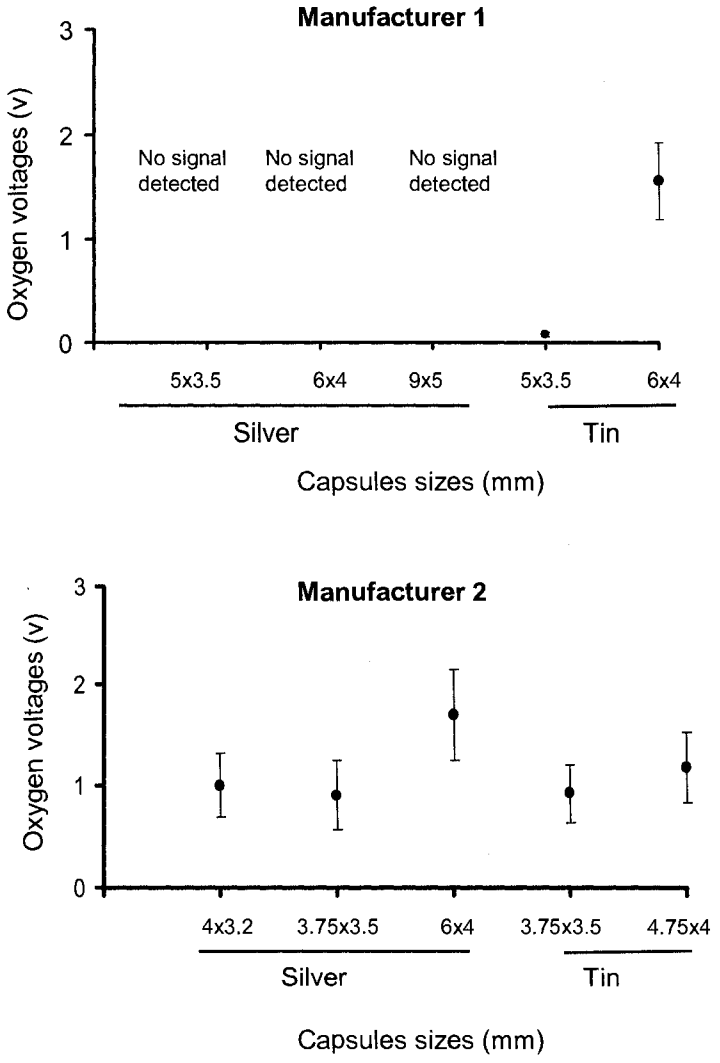

Fig. 2.2 Blank signals (volts) detected from capsules of various sizes and materials from manufacturer 1 and 2.

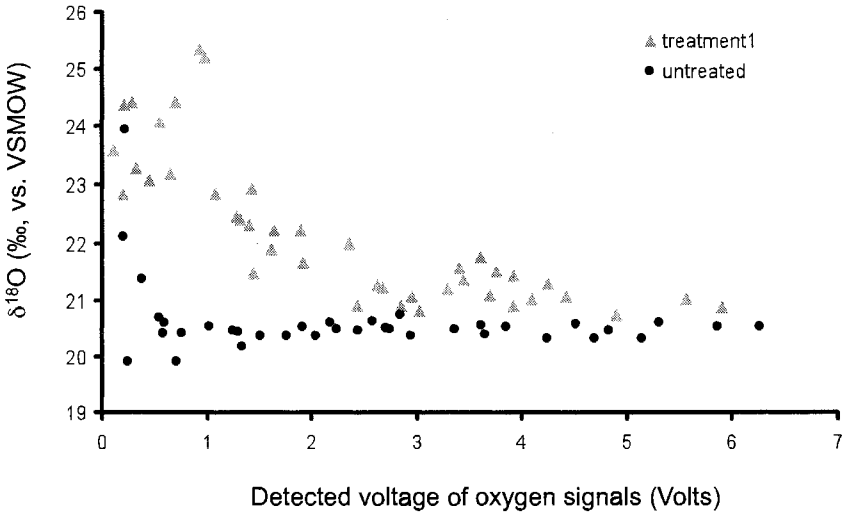

Fig. 2.3 Chitin $\delta^{18}O$ vs. the detected voltages of oxygen signals for samples prepared in untreated 5x3.5mm silver capsules and 5x3.5mm silver capsules rinsed in acetone.

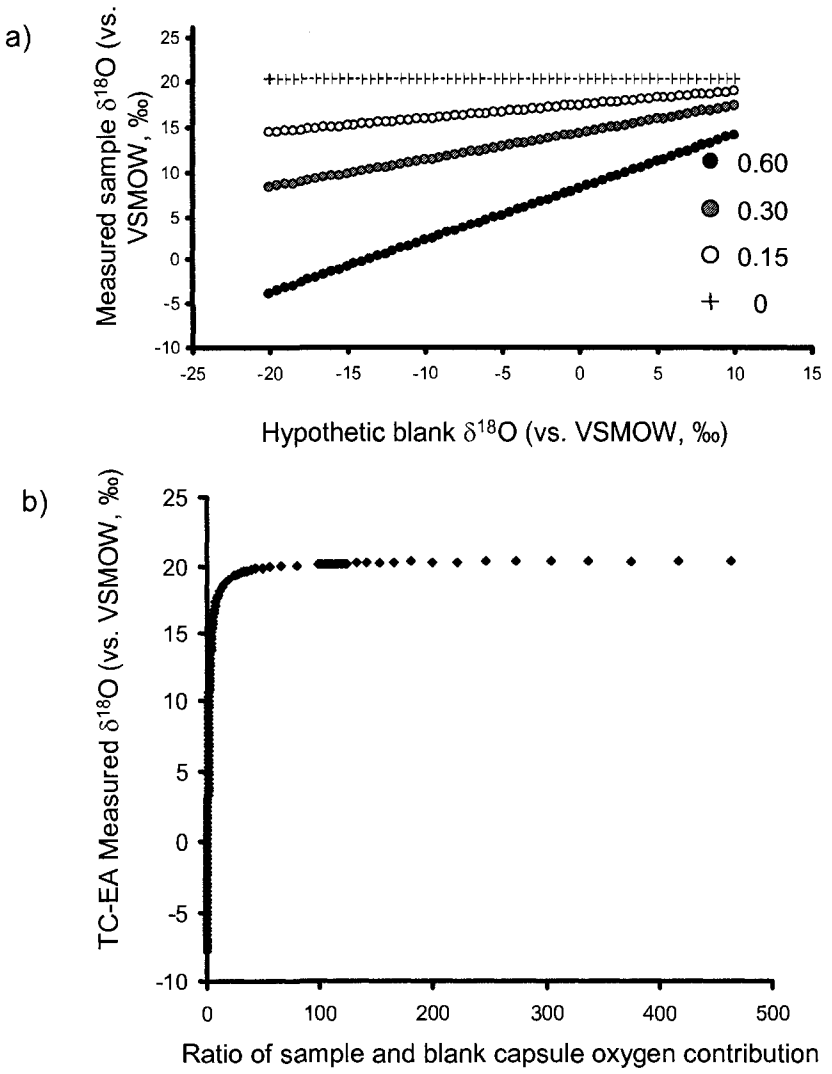

Fig. 2.4 a) A series of mixing models demonstrating the influence of different quantities of blank oxygen on $\delta^{18}O$ of a sample. b) A mixing model of blank oxygen and sample oxygen.

Table 2.1 Average weights, materials, and sizes of capsules from two different manufactures used in the study

Capsule Materials	Manufacturer 1		Manufacturer 2	
	Size (mm x mm)	Weight (mg)	Size (mm x mm)	Weight (mg)
Silver	5x3.5	11.62±0.66 (n=26)	4x3.2	13.80±0.33 (n=10)
	6x4	18.81±0.52 (n=10)	3.75x3.5	13.72±0.48 (n=10)
	9x5	36.87±1.38 (n=10)	6x4	28.52±0.61 (n=10)
Tin	5x3.5	10.21±0.12 (n=10)	3.75x3.5	5.70±0.06 (n=10)
	6x4	15.29±0.28 (n=10)	4.75x4	8.19±0.11 (n=10)

Table 2.2 Results from empirical tests of capsule types from two different manufacturers

Manufacturer	Capsule materials	Sizes (mm x mm)	Treatment	Detected blank oxygen voltages	Detected blank hydrogen voltages
1	Silver	5x3.5	Unrinsed	0 (n=16)	0 (n=16)
			Ultrapure water rinsed	0 (n=16)	0 (n=16)
		6x4	Unrinsed	0 (n=10)	0 (n=10)
			Ultrapure water rinsed	0 (n=10)	0 (n=10)
		9x5	Unrinsed	0 (n=10)	0.02±0.01 (n=10)
			Ultrapure water rinsed	0 (n=10)	0.02±0.01 (n=10)
	tin	5x3.5	Unrinsed	0.08±0.02 (n=20)	0 (n=20)
			Ultrapure water rinsed	0.09±0.02 (n=20)	0.01±0.03 (n=20)
		6x4	Unrinsed	0.12±0.03 (n=20)	0.08±0.01 (n=20)
			Ultrapure water rinsed	0.14±0.05 (n=20)	0.12±0.09 (n=20)
2	Silver	4x3.2	Unrinsed	1.00±0.03 (n=14)	0 (n=14)
			Ultrapure water rinsed	0.68±0.49 (n=10)	0 (n=10)
		3.75x3.5	Unrinsed	0.91±0.34 (n=18)	0 (n=18)
			Ultrapure water rinsed	0.50±0.64 (n=10)	0.02±0.07 (n=10)
		6x4	Unrinsed	1.70±0.45 (n=15)	0 (n=15)
			Ultrapure water rinsed	Not tested	Not tested
	tin	3.75x3.5	Unrinsed	0.92±0.28 (n=18)	0 (n=18)
			Ultrapure water rinsed	0.66±0.37 (n=10)	0 (n=10)
		4.75x4	Unrinsed	1.18±0.35 (n=20)	0 (n=20)
			Ultrapure water rinsed	Not tested	Not tested

Table 2.3 Chitin $\delta^{18}O$ prepared in 5x3.5mm silver capsules rinsed in acetone and untreated 5x3.5mm silver capsules.

	Treatment 1*	Untreated
Mean $\delta^{18}O$ (all samples)	22.2±1.3 (n=42)	20.6±0.7 (n=37)
Mean $\delta^{18}O$ (voltages >0.5v)	21.9±1.2 (n=36)	20.5±0.2 (n=33)

* Treatment 1= rinsed in acetone.

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CHAPTER 3

The influence of diet and water on the stable oxygen and hydrogen isotope composition of aquatic organisms (Chironomidae: Diptera) with paleoecological implications[1]

Abstract

Stable oxygen and hydrogen isotope analyses of fossil aquatic organisms (- the chitinous head capsules of chironomid larvae) are promising proxies for inferring paleoecological conditions. For $\delta^{18}O$ and $\delta^{2}H$ data from analyses of fossil chironomid chitin to be used effectively in paleoecological research, it is necessary to understand the factors controlling the stable oxygen and hydrogen composition of chironomids. We cultured chironomid larvae (Chironomidae: Diptera) in two isotopically distinct waters (natural abundance water $\delta^{18}O$ = -15.1±1.2 ‰, $\delta^{2}H$ = -123.9±7.9 ‰ and enriched water $\delta^{18}O$ = 6.5±0.7 ‰, $\delta^{2}H$ = 35.9±16.0 ‰) under controlled, replicated laboratory conditions. Chironomid larvae were fed on identical diets, to examine the degree to which water and diet influence the $\delta^{18}O$ and $\delta^{2}H$ of these organisms. We used a two-end member mixing

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[1]Wang, Y. O'Brien, D. M. Jenson, J., Francis,D., Wooller, M. J., The influence of diet and water on the stable oxygen and hydrogen isotope composition of aquatic organisms (Chironomidae: Diptera) with paleoecological implications. Prepared for submission to the Proceedings of the National Academy of Sciences (PNAS)

model to determine the proportional contributions of oxygen and hydrogen from water and diet to the oxygen and hydrogen of chironomid larvae. Our experiment demonstrated that 69.0±0.4 % of oxygen and 30.8±2.6 % of hydrogen in chironomid larvae are derived from habitat water. Our results show that oxygen isotopes from chironomid remains can better constrain past habitat water isotopic changes compared to hydrogen, due to 69 % of the chironomid oxygen being influenced by habitat water. Our data also add to a growing suite of comparative data derived from analyses of the proportional contribution of oxygen and hydrogen from diet and water to the organic composition of organisms.

Introduction

Stable oxygen and hydrogen isotope data derived from analyses of animal remains preserved as fossils have been used as proxies of past environmental changes (1-8). Two assumptions in all these studies are that organisms record the isotopic composition of their source waters in their organic compounds and that the source waters are primarily derived from precipitation (9-13). Storm-track trajectories, moisture origins, seasonality, and climate conditions can result in substantial temporal gradients in the stable isotopic compositions ($\delta^{18}O$ and $\delta^{2}H$) of precipitation (9-12). The stable isotopic composition of past precipitation has been examined using various fossil materials such as teeth, bones and the chitinous exo-skeletons of animal remains (8, 14-16). For example, $\delta^{18}O$ analyses of tooth enamel have been used to reconstruct past aridity (8), while $\delta^{18}O$ and $\delta^{2}H$ analyses of deer bones have been used to reconstruct past humidity (5, 6). $\delta^{2}H$ of beetle

chitin preserved in lake sediments has also been used to reconstruct past precipitation changes (1).

Stable isotope analyses of subfossil chironomid head capsules preserved in lake sediments are a recent addition to the suite of tools used to reconstruct the stable isotopic composition of past lake water (4, 17, 18), a proxy for assessing past precipitation patterns and hydrological history. Chironomid head capsules, which are composed primarily of chitin, are well preserved in lake sediments (19) and the stable isotope (carbon, nitrogen, oxygen and hydrogen) composition of chitin is not significantly altered by biological and thermal degradation (20). The family Chironomidae is one the most abundant and widespread aquatic insects, and even chironomids are able to live in extreme environments such as those in Antarctica (21, 22). Changes in chironomid faunal assemblages over time have been used to infer past environmental changes (i.e. temperature, salinity and oxygen availability) based on modern calibration data sets and transfer functions (23-26). The stable oxygen and hydrogen isotope analyses of chironomid chitin can provide supplemental information to reconstruct the past ecological conditions using the same organisms (4, 18).

For $\delta^{18}O$ and $\delta^{2}H$ data from analyses of fossil chironomid chitin to be used effectively in paleoecological research, it is necessary to understand the factors controlling the stable oxygen and hydrogen composition of chironomids (2, 17). Although the $\delta^{18}O$ of habitat water seems to have a strong influence on the $\delta^{18}O$ of chironomids (13), diet may also play a role in influencing the isotopic composition of

chironomids (1, 20, 27). The degree to which water and diet influence the $\delta^{18}O$ and $\delta^{2}H$ of aquatic organisms is unknown.

A handful of previous studies have demonstrated that the proportional contribution of hydrogen derived from water and diet to the composition of organisms is fairly consistent among different organisms (7, 10, 12, 28, 29). In contrast, the proportional contribution of oxygen derived from water and diet to the composition of organisms varies greatly among different organisms (7, 10, 12, 28, 29). For example, the hydrogen in human hair (keratin), feathers of Japanese quails, and the organic composition of microbes was found to derive between 26 % and 36 % from drinking or habitat water. In contrast the proportional contribution of oxygen from drinking water varied from 27 % in humans to 70 % in microbes (7, 10, 12, 28, 30). It is possible that aquatic organisms like chironomids may be more strongly influenced by the isotopic signatures of their habitat water. However, the influences of water and diet on the stable oxygen and hydrogen isotope composition of aquatic organisms are largely unexplored and controlled experiments are necessary to understand the factors influencing the stable isotopic composition of organisms. Chironomids provide an excellent model for examining the influence of environmental parameters on their isotopic composition because they can be cultured under controlled laboratory conditions (31).

Here we present the results of a controlled, replicated growth experiment designed to quantify how the $\delta^{18}O$ and $\delta^{2}H$ of water and diet influence the $\delta^{18}O$ and $\delta^{2}H$ of chironomid larvae. We grew chironomid larvae on an isotopically homogeneous diet, in

two waters of contrasting $\delta^{18}O$ and $\delta^{2}H$ signatures. We then calculated the proportional contribution of oxygen and hydrogen from water and diet to larval tissue.

Materials and methods

Study species and culture setup

Chironomus dilutus were supplied as egg masses by the Environmental Protection Agency (EPA Mid-continent Ecology Division, Duluth, Minnesota). This species has been used for toxicity tests by the U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Center, and the EPA, and its rearing requirements are well known (such as food types, growth temperature, growth rate and oxygen requirements). The life cycle of *C. dilutus* is between 4 to 6 weeks at 20-23 °C under *ad libitum* feeding conditions. Our experiment was conducted in an environmental chamber (R.W. Smith & Co, San Diego, CA) at the Water & Environmental Research Center (WERC) at University of Alaska Fairbanks (UAF). Our culture operation was based on the EPA (Mid-continent Ecology Division) standard operating procedures (32) and our culture set up is shown in Fig. 3.1.

C. dilutus were cultured in clear plastic aquaria (28x16x20 cm), each containing five liters of water. Aquaria were maintained at 23 °C with a photoperiod of ~16L:8D. A ~2 cm layer of fine silica sand (<0.2 mm grain size), pre-combusted for 24 hours at 200 °C, was provided as a substrate. The aquaria stood in a water bath (23 °C) leveled

with gravel to help buffer any fluctuations in environmental temperature. Each aquarium was aerated using an air-stone to maintain high levels of dissolved oxygen (DO) throughout the entire experiment (DO levels were >90 % in the aquaria measured on a calibrated YSI 556 DO meter). One liter of water was replaced each day via a gravity feed system from a 1 L Nalgene bottle, and drained through a drainage hole 11 cm from the bottom of the aquarium (Fig. 3.1). This minimized the concentration of ammonium and the decomposition of excessive food in the aquaria. The pH, ammonium levels, and ionic content of the water in each aquarium were monitored daily throughout the experiment to make sure they remained constant.

Experimental design

The experiment ran for ~8 weeks, determined by the larval development time. Two and one half egg masses were placed into each aquarium, and chironomid larvae were allowed to develop from eggs to fourth instar larvae (determined by body length measurements following the EPA chironomid culturing standard operating practice) (32). Once the larvae had reached their fourth instar, they were harvested using a pair of fine forceps and stored in a freezer until their analysis. Larvae from all of the aquaria were terminated at the same time. The numbers of chironomid larvae harvested from each aquarium were not the same, which may have resulted from variability in hatching success rate for each aquarium due to inadequate egg mass fertilizations (33).

Two water treatments with different stable isotopic compositions [natural abundance (W_1) and isotopically labeled (W_2)] were used in this experiment, with three aquaria for each treatment (n = 6 aquaria). Water was collected from the Fox spring outside of Fairbanks, AK in March 2007 in two 200 L drums, and kept at 4 °C throughout the experiment. W_1 consisted of natural abundance Fox spring water in one of the drums. W_2 consisted of Fox spring water in the second drum that was isotopically labeled using water enriched in ^{18}O (>97 % atom percent) (Iso-Solution Inc., Ottawa, CA) and deuterium oxide (^2H >99.9 % atom percent) (Cambridge Isotope Laboratories, Andover, MA). Ten milliliters of ^{18}O enriched water and 6.5 ml enriched deuterium oxide were added to W_2. The δ^2H of W_1 and W_2 was -140.0±3.6 ‰ and 60.0±3.6 ‰, respectively; the $\delta^{18}O$ of W_1 and W_2 was -17.5±0.6 ‰ and 9.6±0.3 ‰, respectively.

The diet for each treatment was constant throughout the experiment and consisted of fine powdered *Spirulina* algae (Aquatic Eco-Systems Inc.). Ten grams of dry *Spirulina* algae was mixed with 500 ml of water from each treatment and food mixes were stored in the refrigerator for use during the experiment. A final concentration of 0.02 mg/mL was achieved for each aquarium each day. Feeding stopped a week before harvesting to minimize larval gut contents.

Isotope sampling and analysis

Stable isotope ratios are expressed in "delta" notation (δ) in per mil (‰): δ (‰) = ($R_{sample}/R_{standard}$-1) x1000 with R being the ratio between the abundances of heavy to light

isotopes (e.g. $^{2}H/^{1}H$, $^{18}O/^{16}O$) for the sample and standard. The isotope compositions of our results are expressed relative to international standards [Vienna Pee Dee Belemnite (VPBD) for carbon, atmospheric nitrogen (air) for nitrogen and Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen].

We sampled water from each aquarium at two-day intervals throughout the experiment to check that the isotopic composition of the growth water remained constant. We did not use lids for the aquaria and some evaporation from the aquaria did occur during the experiment. Water samples were collected into a 2 ml glass vial by pipette and were then crimped shut with no headspace in preparation for stable isotopic (O and H) analysis. The vials were loaded into an autosampler (CTC Analytics A200SE liquid autosampler) and 0.2 μl of each sample was injected into an on-line pyrolysis, thermochemical reactor elemental analyzer (Finnigan ThermoQuest TC/EA) coupled to a continuous flow (conflo III) isotope ratio mass spectrometer (IRMS) (Finnigan MAT Delta V) at Alaska Stable Isotope Facility (ASIF) at the UAF. The quality control scheme involved analyzing laboratory working standards after every seventh sample. Laboratory working standards were internally calibrated Duckering Building Millipore Water (DMW), NIST (REF 8535 VSMOW), GISP (8536) and SLAP (8537) and measured vs. expected had an R^2 of >0.99. Multiple (n = 15) $\delta^{18}O$ and $\delta^{2}H$ analyses of DMW conducted during the sample sequence yielded 1σ = 0.4 ‰ and 1.7 ‰ respectively. Each sample and standard was analyzed in triplicate. Triplicate $\delta^{18}O$ and $\delta^{2}H$ analyses of separate DMW and water samples yielded 1σ of $\leq$0.3 ‰ and 1.6 ‰ respectively.

The $\delta^{18}O$ and $\delta^{2}H$ of larvae and diet were also measured at the ASIF. Between 0.1 and 0.3 mg of individual freeze dried chironomid larvae and diet samples were weighed into tin capsules. Acknowledging the possibility of exchangeable hydrogen in organic samples (2, 20, 27-29), we adopted a method consistent with that outlined by Wassennar and Hobson (34), where samples were air equilibrated with ambient laboratory air moisture in the ASIF at room temperature for >96 hours prior to freeze drying for >6 days (as recommended for organic samples such as keratin (35) to minimize the influence of exchangeable oxygen and hydrogen). Moreover, we treated all of our samples in the same manner allowing relative comparisons between samples. After comparative equilibration and freeze drying, all samples were loaded into a zero-blank autosampler that was purged with research grade Helium. Measurements of the $\delta^{18}O$ and $\delta^{2}H$ of these organic samples were made using an online pyrolysis thermochemical reactor (Finnigan ThermoQuest TC-EA) coupled with a conflo III and Thermo Finnigan Delta V IRMS. $\delta^{18}O$ and $\delta^{2}H$ of gases (CO and H_2) from each sample were measured relative to calibrated reference gases (CO and H_2). $\delta^{18}O$ and the $\delta^{2}H$ of sample gases were then calibrated relative to internally calibrated organic standards BWBII (34) and international, calibrated standards (ANU sucrose, NBS 22, NBS 30 and PEF 1) (measured vs. expected R^2 = >0.99). The $\delta^{18}O$ and $\delta^{2}H$ analyses of laboratory working organic standards, Benzoic acid (Fisher Scientific, Lot No 947459), were also conducted throughout the run (n = 10) and yielded 1σ of 0.4 ‰ and 1.74 ‰ respectively. Blank tin capsules were also analyzed among the samples.

To assess whether chironomid larvae ate only the diet we fed to them, we analyzed the $\delta^{13}C$ and $\delta^{15}N$ of food (*Spirulina*) and chironomid larva in a initial experiment in 2006. These larvae were freeze dried before weighing and analyzed for $\delta^{13}C$ and $\delta^{15}N$. Microorganisms were observed growing on the inner wall of the aquarium and these were also collected and analyzed for $\delta^{13}C$ and $\delta^{15}N$. The $\delta^{13}C$ and $\delta^{15}N$ values of the samples were determined using a Costech ECS4010 Elemental Analyzer (EA) attached via a Conflo III to a continuous flow IRMS (Thermo Finnigan Delta^{plus} XL) at the ASIF. Multiple (n = 7) analyses of our laboratory working standard (Peptone, Sigma Inc. Lot No. 76F-0300) yielded 1σ of 0.1 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. The second and fourth larval instars were also measured for $\delta^{18}O$ and $\delta^{2}H$ using the procedure described above to examine whether the isotopic composition varied between larval stages.

Data analysis

A mixing model was used to calculate the proportion of diet and water contributing to the oxygen isotope composition of chironomids:

$$\delta^{18}O_C = (\delta^{18}O_D + \varepsilon_D)(1-p) + (\delta^{18}O_W + \varepsilon_W)p \qquad [1]$$

Where C = chironomid, D = diet, W = water, and p = the proportional contribution of water to chironomid tissues. The terms ε_D and ε_W are fractionation effects associated

with diet and water uptake, respectively. Because $\delta^{18}O_D$ and ε_D are the same for both treatments, we can solve for p by using the two experimental water types as follows:

$$p = \frac{(\delta^{18}Oc_{(W1)} - \delta^{18}Oc_{(W2)})}{(\delta^{18}O_{W1} - \delta^{18}O_{W2})} \qquad [2]$$

Where W_1 is the natural abundance water and W_2 is the isotopically labeled water. The same equations can also be used to calculate the proportional contributions of hydrogen from diet and water to that of chironomids by substituting H into the equations where O is used. The possible contribution of atmospheric oxygen to the oxygen isotope composition of the chironomid larvae in both treatments were assumed constant for all aquaria and a constant offset would be expected.

Statistical analyses

All statistical analyses were performed using JMP IN 5.2.1 (JMP, SAS Institute, Cary, NC). Data from the two treatments were analysed separately. Values of $\delta^{18}O_C$ (δ^2H_C) and $\delta^{18}O_w$ (δ^2H_w) were analyzed using ANOVA to compare among tanks within two treatments. We assessed the differences among chironomid larvae and water from different aquaria with Turkey HSD contrasts. Because calculations of p required the $\delta^{18}O_C$ (δ^2H_C) to be compared with the $\delta^{18}O_w$ (δ^2H_w), we used the mean of the $\delta^{18}O_C$ (δ^2H_C) compared with the $\delta^{18}O_w$ (δ^2H_w) (Eq. 2) of each tank. All these measurements

are reported as the mean ± 1 standard deviation (mean ± 1SD). Brown and Forsythe's tests were performed to check the constant variances of $\delta^{18}O_C$ and δ^2H_C within each treatment. Isotope differences in two larvae stages were assessed using a Student T-test. Unless otherwise stated, all statistical test results refer to 95% significant intervals.

Results

Stable isotopic composition of water

There were no significant differences in $\delta^{18}O_W$ and δ^2H_W among the three natural abundance aquaria ($p = 0.211$ for $\delta^{18}O$ and $p = 0.235$ for δ^2H) (Table 3.1). Labeled water treatments were heavily enriched relative to the natural abundance treatments throughout the entire experiment. However, $\delta^{18}O_W$ and δ^2H_W were not identical between aquaria, which may have been due to slight differences in evaporation levels from each aquarium. The differences were observed among three isotopically labeled water aquaria for both $\delta^{18}O_W$ and δ^2H_W (ANOVA, $p = 0.007$ for both $\delta^{18}O$ and δ^2H), and a post hoc contrast test showed that there was a significant difference between aquarium 4 and 5 ($p<0.05$ both $\delta^{18}O$ and δ^2H).

Stable isotopic composition of chironomid larvae

Similar to the water, the $\delta^{18}O$ and $\delta^{2}H$ of chironomid larvae from the aquaria were distinctly different between the natural abundance (W_1) and enriched water treatments (W_2) (Table 3.1, Fig. 3.2). However, the magnitude of the difference between the two treatments is much smaller compared to that of their growth water (Fig. 3.2). This is a clear indication that diet also influenced the $\delta^{18}O_C$ and $\delta^{2}H_C$ of the larvae. Variation in $\delta^{18}Oc$ and $\delta^{2}Hc$ among aquaria were also observed within the same treatment; however, isotopic variability of larvae within the same water treatment was much smaller than the variability between the two growth water treatments. Differences in the larvae grown in W_1 aquaria were significant for $\delta^{18}O$ but not for $\delta^{2}H$ ($p = 0.01$ and $p = 0.33$ for $\delta^{18}O$ and $\delta^{2}H$ respectively) and the Turkey HSD test was only significant between aquaria 2 and 3 ($p<0.05$). The variances among natural abundance aquaria were constant for the larvae $\delta^{18}O$ and $\delta^{2}H$ ($p = 0.41$ for both). The larvae grown in the W_2 did not show significant differences for their $\delta^{18}O$ and $\delta^{2}H$ ($p = 0.34$ for $\delta^{18}O$ and $p = 0.53$ for $\delta^{2}H$) and variances among aquaria were also constant ($p = 0.09$ and $p = 0.63$, for $\delta^{18}O$ and $\delta^{2}H$ respectively).

Proportional contributions of oxygen and hydrogen from water vs. diet to chironomid larvae

We used two approaches to calculate the proportional contributions of oxygen and hydrogen from water and diet to the organic composition of chironomid larvae. In the first approach, by neglecting the slight differences among replicate aquaria within the same treatment, we calculated the mean $\delta^{18}O$ and $\delta^{2}H$ of all water samples measured for each treatment (W_1 and W_2) and the mean $\delta^{18}O$ and $\delta^{2}H$ of chironomids associated with each treatment (Table 3.1). By using these data to solve equation [2], we found that 72 % of chironomid oxygen was derived from water whereas 32 % of their hydrogen is derived from water. This result is represented graphically in Fig. 3.3 where the slopes of the relationship between water and chironomid isotope values represent p. However, because there were small but significant isotopic differences between the water in replicate aquaria 4 and 5 and also among the $\delta^{18}O$ for chironomid larvae in replicate aquaria 2 and 3, we also solved equation [2] for each individual aquarium. In order to do this, we calculated the proportional contribution (p) of oxygen and hydrogen from water for every possible paired combination of natural abundance (three) and enriched aquaria (three) (a total of nine combinations). We then solved equation [2] and calculated the p for both oxygen and hydrogen from the nine combinations and the mean p from these 9 combinations (Table 3.2). Using this approach we estimated that 69±4 % of oxygen in chironomid larvae was derived from the water they lived in whereas 31±3 % of hydrogen in chironomid larvae was derived

from water. In both approaches, we calculated the proportional contribution using the mean chironomid $\delta^{18}O$ and $\delta^{2}H$ for each aquarium, because more than 100 chironomid heads are used for a single isotope value in paleo-research (17). These two approaches yielded very similar results: that only ~30 % of oxygen in chironomid larvae originated from diet while ~70 % of hydrogen in the larvae was derived from diet.

Diet of chironomid larvae from the initial experiment

Spirulina (n = 3) (hereafter: diet) yielded a mean $\delta^{13}C$ and $\delta^{15}N$ of -25.8±0 ‰ and -1.4±0.5 ‰, respectively. Whereas the chironomids (n = 3) yielded a mean $\delta^{13}C$ and $\delta^{15}N$ of -25.1±0.2 ‰ and 2.0±0.1 ‰, respectively. The microorganisms collected from the walls of the aquarium had a distinctly different $\delta^{13}C$ and $\delta^{15}N$ values of -34.4±2.1 ‰ and -6.0±1.5 ‰ compared to the diet. The larvae had mean $\delta^{13}C$ and $\delta^{15}N$ values that were 0.7 ‰ and 3.4 ‰ respectively higher than the mean composition of diet in the aquarium (Fig. 3.4). The $\delta^{18}O$ and $\delta^{2}H$ of the diet was 28.0±0.9 ‰ and -152.3±2.6 ‰, respectively and the $\delta^{18}O$ and $\delta^{2}H$ analyses of second (n = 3) and fourth larval instars (n = 3) showed that there was no significant difference between these life stages ($p = 0.781$ and $p = 0.744$ for $\delta^{18}O$ and $\delta^{2}H$ respectively).

Discussion

The $\delta^{18}O_C$ and $\delta^{2}H_C$ from two water treatments were distinctly different from each other but with little variation within each treatment, despite being fed the same diet. These differences are consistent with the $\delta^{18}O_W$ and $\delta^{2}H_W$ of their growth water. However, the magnitude of differences between chironomid larvae from the two treatments is smaller than that of two growth water. These results demonstrate that both water and diet affect the oxygen and hydrogen isotope composition of chironomid larvae. By providing a diet with constant isotopic composition and water with two different isotopic compositions, we were able to quantify the proportional contributions of oxygen and hydrogen from water to chironomid larvae. Our data show that ~70 % of the oxygen in the total organic composition of chironomids is derived from the water of the larval habitat. In contrast, diet dominates the hydrogen isotope ratios of chironomid larvae (~70%) because only 30 % of hydrogen in chironomids was derived from water during larval development. Interestingly, our findings for both oxygen and hydrogen are identical to the proportional contributions of oxygen and hydrogen from water and growth substrate supplied to microbial spores, where 70 % of oxygen and 30 % of hydrogen of microbial spores were found to derive from water, whereas the remainder was derived from the organic compounds supplied as substrate (28).

Our finding that ~30 % of chironomid total organic hydrogen derives from water is consistent with the handful of published studies of animals, both observational and experimental. Despite differences in tissues, the influence of water is remarkably

consistent. Estimates of the drinking water contribution to hair hydrogen in humans range from 27 to 35 % (10, 12, 30). Similarly, the contribution of water to hydrogen in bird feather keratin was between 26 and 32 % (7). These results confirm that the majority of the hydrogen in lipids, protein and keratin are derived from diet rather than water. The hydrogen metabolic pathway responsible for this process is unknown. Hobsen et al., (7) speculated that it possibly involves hydrogen in drinking water exchanging with hydrogen from macro-molecules in the stomach content.

Our estimate that ~70 % of total organic oxygen in chironomid larvae derived from water is about twice as high as has been reported in two previous human studies (10, 12). It is not yet clear why proportional contributions of oxygen from water to chironomids (this study) and microbes (28) are different from humans. Even though they use hemoglobin in anoxic conditions, chironomid larvae uptake oxygen primarily by gas diffusion from the surface of their body (36). The difference in proportional contribution of oxygen from water to chironomids and microbes compared with humans may be related to their different metabolic pathways of O_2 uptake and loss.

To assess the consistency of our results, we plotted the mean $\delta^{18}O$ and $\delta^{2}H$ values of chironomids and growth water from both the preliminary culture trial and the main experiment (Fig. 3.3). The preliminary $\delta^{18}O$ data did not change the slope of the regression line from the main experiment (Fig. 3.3a). However, the preliminary $\delta^{2}H$ data shifted the slope from 0.31 to 0.35 (Fig. 3.3b) and while not exactly identical, the data are consistent with our estimated proportional contribution of water to chironomid total organic hydrogen (31±3 %).

Chironomids seemed only to have consumed one diet during the entire experiment since the $\delta^{13}C$ and $\delta^{15}N$ of chironomid to diet fractionations ($\varepsilon\delta^{13}C = 0.7$ ‰ and $\varepsilon\delta^{15}N = 3.4$ ‰) (Fig. 3.4) are consistent with a classic trophic level fractionation ($\varepsilon\delta^{13}C = \sim 1$ ‰, $\varepsilon\delta^{15}N = \sim 3$ ‰) (37, 38). The $\delta^{13}C$ and $\delta^{15}N$ values of microorganisms found growing on walls of the aquarium were distinctively different from those of the diet, which shows that the microorganisms were a very unlikely source contribution to the diet of the chironomids. Thus our calculations of the contributions of oxygen and hydrogen from diet and water were not effected by alternate diet sources.

Further studies are needed to establish the relationships between specific compounds (e.g. chitin) from chironomids. Previous studies have shown that hydrogen in different tissues (such as muscle, lipids and feathers) in birds can derive from different proportions of hydrogen from water (7). The proportional contribution of oxygen and hydrogen to whole chironomid larvae, which include lipids and proteins, may differ from those of chitin, the predominant material preserved in the fossil record (39). Fractionation factors (ε) associated with both water and diet in our experiment cannot be determined using equation [1] because there are three unknowns (p, ε_w, ε_D). A labeling study of nutritionally identical diets with different isotope ratios would make it possible to solve all the unknowns (40) including the p, ε_w and ε_D. Nevertheless, the differences in the fractionation factor can be observed since the $\delta^{18}O_C$ and $\delta^{2}H_C$ do not lie along a linear relationship established using two mixing sources (Fig. 3.2).

The systematic variation in $\delta^{18}O_C$ and $\delta^{2}H_C$ grown in two significantly different water habitats clearly demonstrates the potential of using $\delta^{18}O_C$ and $\delta^{2}H_C$ of subfossil

chironomids as a tool in paleoecological research. The range of $\delta^{18}O_W$ and $\delta^{2}H_W$ in our experiment is similar to those of natural chironomid habitats. Our findings support that the stable isotopic composition of chironomid subfossils can be used to explain changes in the oxygen and hydrogen isotope values of past growth environments and changes in hydrology and climate (17, 18, 41) can be derived in concert with other geological evidence. The stable oxygen isotope composition of chironomid larvae provides a stronger marker of habitat water isotopic values compared with hydrogen isotopic data derived from chironomids since a greater proportion (~70 %) of chironomid oxygen is derived from water compared with hydrogen (~30 %). Conversely, hydrogen isotope analyses of chironomids better constrain the hydrogen isotopic composition of diet. In our experiment, the magnitude of changes in $\delta^{18}O_C$ and $\delta^{2}H_C$ is based on the mean value of chironomid larvae within each aquarium instead of individual larvae. The systematic variation in each aquarium resembles paleoecological research, where one measurement is based on more than 100 fossil chironomid head capsules. Our research is the first of its type on aquatic invertebrates to calculate the proportional contribution of oxygen and hydrogen from water vs. diet. Our findings add to the growing suite of comparative data available on the proportional contribution of oxygen and hydrogen deriving from water and diet available to organisms.

Acknowledgements

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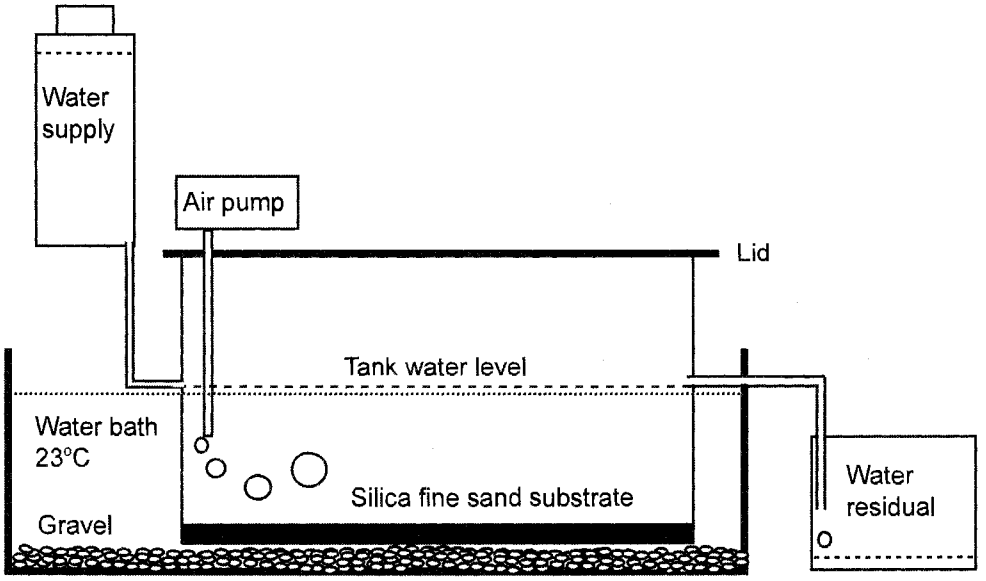

Fig. 3.1 A schematic of the aquarium set-up used to culture chironomid larvae.

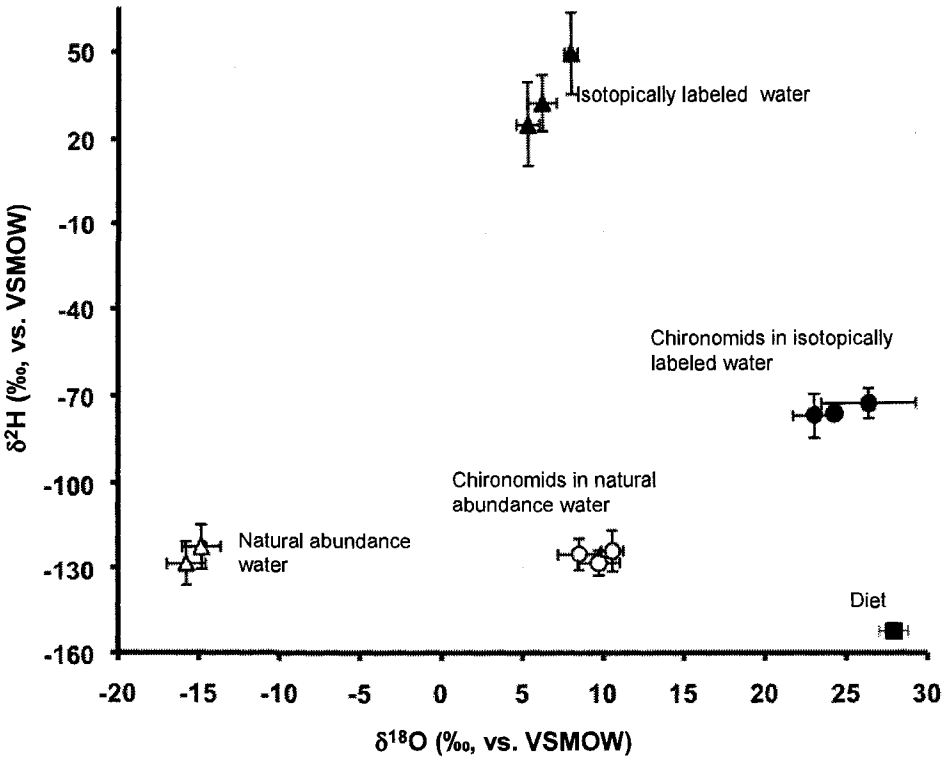

Fig. 3.2 $\delta^{18}O$ and $\delta^{2}H$ of two types of growth water, chironomid larvae from both treatments, and diet. Error bars represent one standard deviation of the mean.

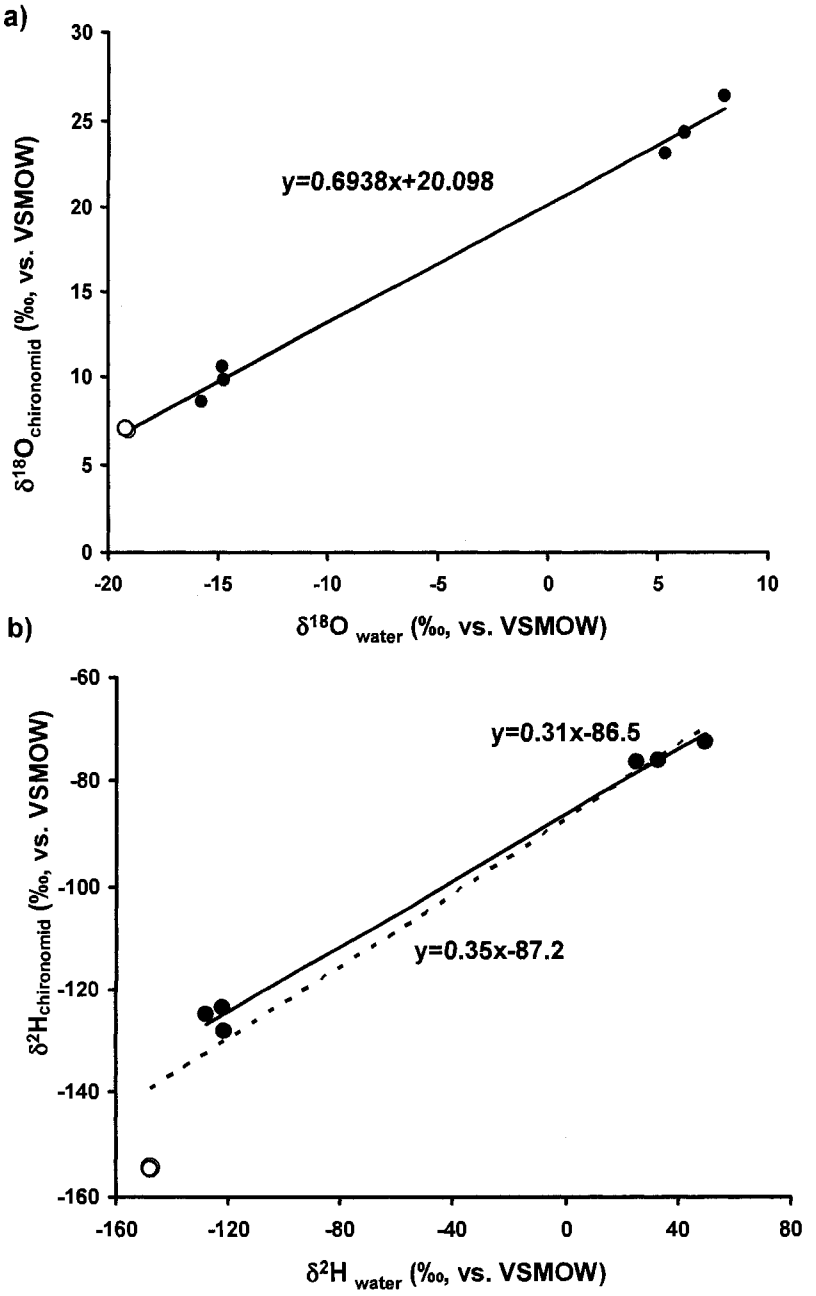

Fig. 3.3 (a) $\delta^{18}O$ values of chironomids vs. their growth water $\delta^{18}O$ (solid circles). (b) $\delta^{2}H$ of chironomids vs. $\delta^{2}H$ of their growth waters (black solid circles). The $\delta^{18}O$ and $\delta^{2}H$ of chironomids vs. water from a separate culture trial is superimposed on the line (open circles). The slope of the regression line changes in (b) when data from the preliminary culture trial is added (dotted line).

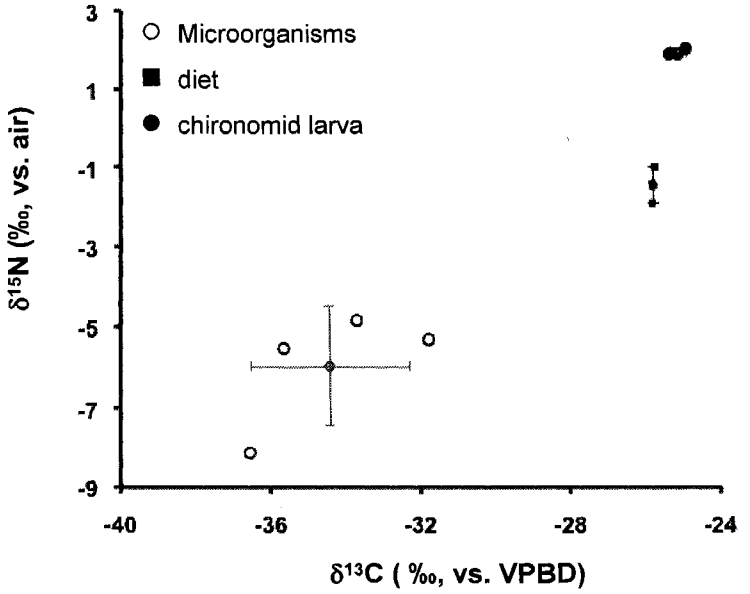

Fig. 3.4 $\delta^{15}C$ and $\delta^{15}N$ of chironomid larvae, their diet (*Spirulina*) and the microorganism growing on the aquarium walls. Error bars represent one standard deviation.

Table 3.1. The $\delta^{18}O$ and $\delta^{2}H$ (mean ± 1SD) of water and chironomid larvae from two water treatments.

Treatment	Aquarium No.	δ^{18}Ow ‰	δ^{2}Hw ‰	δ^{18}Oc ‰	δ^{2}Hc ‰
Natural abundance water	1	-14.7±1.1 (n=7)	-121.5.±7.2 (n=7)	9.8±1.3 (n=10)	-127.8±4.4 (n=10)
	2	-14.8±1.2 (n=7)	-122.1±7.9 (n=7)	10.6±0.7 (n=4)	-123.6±7.3 (n=4)
	3	-15.8±1.2 (n=7)	-128.1±7.9 (n=7)	8.5±1.3 (n=9)	-124.7±5.6 (n=9)
	Mean	-15.1±1.2 (n=21)	-123.9±7.9 (n=21)	9.4±1.3 (n=23)	-125.9±5.6 (n=23)
Isotopically labeled water	4	8.0±1.3 (n=7)	49.6±13.7 (n=7)	26.4±2.9 (n=6)	-72.6±5.1 (n=6)
	5	5.1±1.5 (n=7)	25.3±15.6 (n=7)	23.1±1.3 (n=4)	-76.6±7.5 (n=4)
	6	6.2±1.8 (n=7)	32.7±9.6 (n=7)	24.4±0.2 (n=2)	-76.0±0 (n=2)
	Mean	6.5±0.7 (n=21)	35.9±16.0 (n=21)	24.9±2.6 (n=12)	-74.5±5.6 (n=12)

Table 3.2. The proportional contribution (p) of oxygen and hydrogen derived from water to the organic composition of chironomid larvae.

	***p* of oxygen from water**			***P* of hydrogen from water**		
	Aquaria 1	**Aquaria 2**	**Aquaria 3**	**Aquaria 1**	**Aquaria 2**	**Aquaria 3**
Aquaria 4	0.733	0.692	0.751	0.299	0.273	0.270
Aquaria 5	0.663	0.618	0.688	0.349	0.318	0.313
Aquaria 6	0.695	0.652	0.717	0.336	0.307	0.303
Mean (of 9)	0.690			0.308		
STD	0.041			0.026		

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CHAPTER 4

Variation in the stable oxygen isotopic composition of chironomids as evidence for shifts in atmospheric circulation patterns during the late Quaternary in southwest Alaska[1]

Abstract

Southwest Alaska has been characterized by complex patterns of climate change over the past 16,000 cal yr BP. The stable oxygen and hydrogen isotope analyses of modern precipitation from King Salmon weather station suggest that southwest Alaska receives multiple sources of precipitation during the year. Thus, the $\delta^{18}O$ and δD of lake water in Idavain Lake reflects the proportion of North Pacific moisture (zonal flow) and mixed modern flow (North Pacific moisture and tropical moisture). Analyses of the stable oxygen isotope composition of fossil chironomid head capsules from a sediment core taken from Idavain Lake in southwest Alaska are used as a $\delta^{18}O_{lake\ water}$ proxy and reveal changes in lake water $\delta^{18}O$ during the last 16,000 years. This is the longest record recovered from the region. The large magnitude of variation in $\delta^{18}O_{lakewater}$ are mostly likely due to alternating shifts in the two atmospheric flow regimes that are predominant

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in southwest Alaska. The temporal shifts in flow regime coincide with some changes in other paleoenvironmental proxies from Idavain Lake (i.e. pollen, $\delta^{13}C$, $\delta^{15}N$, C/N). The zonal regime appears to have been dominant from 16,000 to 13,800 cal yr BP, 11,000 to 10,500 cal yr BP, 10,000 to 8,000 cal yr BP, and during a majority of the periods from 8,200 to 3,500 cal yr BP, and from 2,000 cal yr BP to present. A mixed modern flow regime seems to have been dominant during the periods from 13,000 to 11,000 cal yr BP, 10,500 to 10,000 cal yr BP, 6,000 to 5,500 cal yr BP and 2,500 to 1,800 cal yr BP. These possible shifts between zonal and mixed modern flow appear to coincide with a series of glacier advances and recessions along the Gulf of Alaska.

Introduction

Southwest Alaska consists of extensive lowlands and scattered uplands extending from central Alaska to the coast of Bristol Bay and the southern Beringia straits (Fig. 4.1). This region is characterized by a transitional climate condition between maritime and continental climate zones (Bowling, 1979). The complex vegetation history in southwest Alaska since deglaciation suggests a heterogeneous climatic response as a result of changes in atmospheric circulation (Mann et al., 1998; Mann and Hamilton, 1995; Mock et al., 1998; Peteet et al., 1997; Peteet and Mann, 1994). Despite an increase in the number of paleoecological studies in southwest Alaska over the last two decades (Anderson et al., 2006; Brubaker et al., 2001; Hu et al., 1995; Hu et al., 2003; Vlag and Banerjee, 1999), there remains a poor understanding of how effective moisture responds to regional climate controlling mechanisms and processes (e.g. increased insolation during the Holocene and changes in atmospheric circulation patterns) (Brubaker et al., 2001; Finney et al., 2004; Hu and Shemesh, 2003; Kaufman et al., 2004; Peteet et al., 1997). Relatively little is known about moisture availability and atmospheric circulation patterns modulated by radiative forcing during the Holocene (i.e. last ~10,000 years).

In this study, we present results from analyses of sediments from Idavain Lake in southwest Alaska to reconstruct past moisture source changes related to atmospheric circulations. Brubaker et al. (2001) conducted a pollen analysis of a core from this lake that encompassed the last ~16,000 calibrated years before present (cal yr BP), representing the deglaciation. However, pollen records alone are insufficient to determine the specific

mechanisms controlling late-Quaternary (since deglaciation) environmental change in southwest Alaska (Brubaker et al., 2001, Hu and Shemesh, 2003). For this study, a new core was collected and a suite of paleolimnological proxies of past environmental conditions were analyzed, including loss on ignition (LOI), magnetic susceptibility (MS), C/N ratios and stable carbon and nitrogen isotopic analyses ($\delta^{13}C$ and $\delta^{15}N$) of bulk sediments (i.e. total organics). In addition, we conducted stable oxygen and hydrogen isotope ($\delta^{18}O$ and δD) analyses of chironomid head capsules preserved in the sediments. The $\delta^{18}O_{chironomid}$ data from lake sediment cores, taken from other geographic locations have previously been used to reconstruct past $\delta^{18}O_{lakewater}$ (Wooller et al., 2004; Wooller et al., 2007) and examine past moisture conditions and infer changes in atmospheric circulation patterns over time (Wooller et al., 2007).

Study site

Idavain Lake (58°46′N, 155°57′W, 223 m a.s.l.) is located at the southeastern margin of the Nushagak Lowland and is bordered by the Alaska Range to the north and Ahklun Mountain to the west (Fig. 4.1). Mt. Katmai is 90 km to the southeast of Idavain Lake. Idavain Lake is 8 km long and 1.5 km wide, with two deep basins (20 and 21 m water depth) surrounded by extensive flat shelves (ca. 2-5 m water depth) (Brubaker et al., 2001) (Fig. 4.1). The Lake's basin was deglaciated between the 'early phase' of the Brooks Lake and the Newhalen Stades (bracketing 28,000-18,000 and 16,000-14,000 cal yr BP) (Stilwell and Kaufman, 1996). The modern regional vegetation ranges from conifer and/or hardwood

boreal forest in the north and *Betula* shrub tundra with extensive *Alnus* shrubs to the south (Brubaker et al., 2001).

Idavain Lake is approximately 100 km from the Gulf of Alaska (GOA) and the climate in the area is characteristic of a transition zone between continental and maritime climate zones, influenced strongly by westerly winds (Rodionov et al., 2005). King Salmon (58°41′N, 156°39′W, 10 m a.s.l.), is the nearest national weather station (Fig. 4.1) to Idavain Lake, and has a Mean Annual Temperature (MAT) of 0 °C from the period of 1928-2000. The maximum summer temperature often occurs in July, with a mean of 12.7 °C over the period of 1928-2000. The lowest winter temperature is normally in December and January, which both have a mean of -9.7 °C (NCDC, 2004). Total average annual precipitation for the site is ~50 cm/year and the majority of the precipitation falls from July to October, with the highest amounts falling in August (mean of 7.9 cm) (NCDC, 2004).

Certain limnological characteristics of Idavain Lake make it suitable for reconstructing the $\delta^{18}O$ of past precipitation from estimates of the $\delta^{18}O$ of past lake water. The lake water depth is between 8-20 m and the estimated residence time is relatively short (3.3 years) (calculated using equation: residence time=lake volume/inflow) (Langmuir, 1997). Surface inflow is limited to surface run-off from the surrounding watershed and there is only one surface outflow at the southeast end of the lake. The $\delta^{18}O$ of Idavain Lake water was measured in July 2005 as -11.1 ‰, which closely resembles the predicted $\delta^{18}O$ (-12.9 ‰) of mean annual precipitation for the site (estimated from On-line Isotopes in Precipitation Calculator, http://www.waterisotopes.org/) (Bowen, 2008), which indicates that the lake is probably evaporation-insensitive today (Kendall and Coplen, 2001).

Methods

Precipitation and lake water sampling and preparation

We collected precipitation samples from the King Salmon weather station (58°41´N, 156°39´W, 10 m a.s.l.), which is 43 km west of Idavain Lake, to provide a better understanding of the relationship between modern precipitation and lake water from Idavain Lake. A total of 295 precipitation samples were collected by personnel at the King Salmon weather station over the period from August 1st 2006 to July 30th 2007. All water samples were capped with no headspace after collection and stored in a refrigerator. Samples were mailed to the Alaska Stable Isotope Facility (ASIF) at the University of Alaska (UAF) every two months. A surface lake water sample was collected in a centrifuge tube from Idavain Lake in July 2005 and transported back to UAF. All water samples were transferred into 2 ml glass vials by pipette and then crimped shut with no headspace in preparation for the stable isotopic (O and H) analysis. The vials were loaded into an autosampler (CTC Analytics A200SE liquid autosampler) and 0.2 µl of each sample was injected into an on-line pyrolysis, thermochemical reactor elemental analyzer (Finnigan ThermoQuest TC/EA) coupled to a continuous flow (conflo III) isotope ratio mass spectrometer (IRMS) (Finnigan MAT Delta V) at the ASIF. Quality control included laboratory-working standards after every seventh sample. Laboratory working standards were internally calibrated Duckering Building Millipore Water (DMW), NIST (REF 8535

VSMOW), GISP (8536) and SLAP (8537) and measured vs. expected had an R^2 of >0.99. Multiple $\delta^{18}O$ and δD analyses of DMW (n = 15) conducted during the sample sequence yielded 1σ = 0.4 ‰ and 1.7 ‰ respectively. Each sample and standard was analyzed in triplicate. Triplicate $\delta^{18}O$ and δD analyses of separate DMW and water samples yielded 1σ of $\leq$0.3 ‰ and 1.6 ‰ respectively.

Storm trajectory models helped us examine specific moisture sources at a certain locality, such the King Salmon weather station. By matching isotope values from measurements of precipitation events from this location to storm trajectories, we examined the likely isotopic composition of modern storm events. Storm trajectories were extracted from an online storm track archives at National Oceanic and Atmosphere Administration (NOAA)'s Air Resources Laboratory (ARL) (http://www.arl.noaa.gov/ready.html). Individual storm tracks were matched with their corresponding isotope compositions of precipitation for that period.

Down core sediment laboratory analyses

A 16 m core of sediment was extracted from Idavain Lake with a modified Livingstone piston corer in 2005 and was stored at 4 °C. The amounts of organic carbon in sub-samples taken from along the core were measured as weight loss-on-ignition (LOI) at 550 °C and 850 °C (Bengtsson and Enell, 1986; Dean, 1974) at interval of every 0.5 cm above 16 cm depth, and then at 1 cm interval throughout the rest of the core (total 1498 samples).

Magnetic susceptibility (MS) was measured at the same intervals as the LOI measurements using a Bartington MS meter.

Terrestrial leaf and wood fragments were collected from 11 horizons from the core and were dated at the Lawrence Livermore National Laboratory (LLNL) using Accelerator Mass Spectrometry (AMS). A tephra layer at 16 cm was identified using MS correlation and visual inspection as the widespread tephra layer from the Katmai eruption in 1912 AD (Fierstein, 2007; Riehle et al., 2008). Another tephra layer at 78 cm was cross-dated by correlation with a dated tephra layer (plant macrofossils were dated just below the layer of tephra using AMS at LLNL) from Jo-Jo Lake (58°36′, 155°13′) ~ 47.5 km to the southeast of Idavain Lake.

Bulk sediments from the Idavain core were analyzed at the ASIF for stable carbon and nitrogen. Sediments were not acid washed prior to analysis because no significant amount of carbonates was present (LOI 850°C and Brubaker et al., 2001). A total of 662 sediment samples were taken from the core and analyzed for $\delta^{13}C$, $\delta^{15}N$ and their elemental composition (C % and N %). Above 100 cm the samples were taken at the same horizons as LOI and MS measurements. From 100 to 900 cm the sediments were analyzed at an interval of every 2 cm. Below 900 cm the sediments were analyzed at every 4 cm interval. All stable isotope ratios are expressed in "delta" notation (δ) in per mil (‰) relative to international standards (VPDB for carbon and atmospheric nitrogen for nitrogen). The $\delta^{13}C$ and $\delta^{15}N$ values of the samples were determined using a Costech ECS 4010 Elemental Analyzer (EA) attached via a Conflo III to a continuous flow IRMS (Thermo Finnigan Delta-Plus XL). The one standard deviation derived from 10 analyses

of an internal standard interspersed throughout the run was 0.1 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. The C/N ratios were calculated based on the C % and N % data derived from the EA analyses.

δD and $\delta^{18}O$ analyses of chironomid head capsules

A total of 44 sediment samples (2-4 cc) were taken at 35-45 cm intervals above 1000 cm and 70-80 cm intervals below 1000 cm to extract chironomid head capsules for stable hydrogen and oxygen isotope analyses (δD and $\delta^{18}O$). Four samples (1266 cm, 1026 cm, 982 cm and 344 cm) of the 44 samples processed for chironomids did not yield sufficient sample mass (>0.05mg) for isotope analysis (Wang et al., 2008). No chironomids were extracted from the samples taken at 1580 cm and 1440 cm because the sediments were mostly composed of glacial outwash materials. Samples at depths 156 cm, 398 cm and 870 cm had a large number of chironomid head capsules and were analyzed as triplicates to assess sample precision. The δD and $\delta^{18}O$ analysis of chironomid head capsules were prepared according to the protocol described by Wang et al. (2008). Results were calibrated using an organic standard (calibrated BWBII - Wassenaar and Hobson, 2002) and international, calibrated standards (ANU sucrose, NSS 22, NBS 30 and PEF1). All δD and $\delta^{18}O$ are reported relative to Vienna Standard Mean Ocean Water (VSMOW). Analytical precisions for δD and $\delta^{18}O$, determined as one standard deviation calculated from multiple analyses (n = 9) of an internal laboratory standard (benzoic acid, Fisher Scientific, Lot No. 947459), were 3.2 ‰ and 1.3 ‰, respectively. The triplicate δD and

$\delta^{18}O$ analyses of chironomid head capsules from the same sediment depths produced one standard deviations for δD = 0.9 ‰ and $\delta^{18}O$ = 1.4 ‰ at depth 156 cm, δD = 1.2 ‰ and $\delta^{18}O$ = 1.5 ‰ at depth 398 cm, and δD = 5.8 ‰ and $\delta^{18}O$ = 1.6 ‰ at depth 870 cm. These values are consistent with the analytical precisions based on multiple analyses of benzoic acid reported above. We therefore conservatively use the mean of these triplicates as our estimate of sample precision (δD = 3.2 ‰ and $\delta^{18}O$ = 1.5 ‰) for analysis of chironomid head capsules.

Results

Stable isotope hydrology of Idavain Lake

There was a large variation in the δD and $\delta^{18}O$ values of precipitation (n=295) from King Salmon weather station, ranging from -240.0 to -26.0 ‰ and -33.0 to -1.5 ‰ respectively (Fig. 4.2). Individual rain events within a month, such as August for example, varied from -155.0 to -21.5 ‰ for δD and from -20.2 to -1.5 ‰ for $\delta^{18}O$. A similar magnitude of variation between individual rain events was also evident in the other months. The slope (~6) of the King Salmon's Local Meteoric Water Line (LMWL) is slightly less than the slope (8) of the Global Meteoric Water Line (GMWL) (Rozanski et al., 1993) (Fig. 4.2). The volume weighted δD and $\delta^{18}O$ data from the precipitation collected from King Salmon weather station from August 2006 to July 2007 also show considerable monthly and seasonal variability in the region (Fig. 4.3a). The lowest isotopic values occurred in

December 2006, yielding a mean δD of -171.3 ‰ and $\delta^{18}O$ of -23.8 ‰. The highest isotopic values of precipitation occurred in July 2007 and October 2006 and both months had a mean δD of -87.3 ‰ and $\delta^{18}O$ of -10.7 ‰ (Fig. 4.3a), which are also consistent with the previously measured lake water values.

The δD and $\delta^{18}O$ of Idavain lake water (δD= -86.7 ‰ and $\delta^{18}O$=-11.1 ‰) measured in July 2005 superimposed directly on the LMWL from King Salmon (Fig. 4.2). Notably, the peak precipitation season (July to October) yielded a mean δD of -92.1 ‰ and $\delta^{18}O$ of -11.6 ‰ respectively, which are almost identical to the previously measured lake water values. Whereas the non-peak precipitation season (November to June) yielded a volume-weighted average (= Σ (volume x $\delta^{18}O$)/ Σ (volume)) of -124.0 ‰ and -16.0 ‰ for δD and $\delta^{18}O$ respectively. The temperature and amount of precipitation at King Salmon weather station tracked each other (Fig. 4.3b), whereas the δD and $\delta^{18}O$ of precipitation do not clearly follow the changes in temperature and the amount of precipitation (Fig. 4.3a), indicating that the isotope composition of precipitations is not significantly correlated with temperature and precipitation amount (R^2=0.5 and R^2=0.1, respectively).

Storm trajectories extracted from an online storm track archive at NOAA's Air Resources Laboratory (ARL) (Fig. 4.4) demonstrated that King Salmon receives its precipitation sources from several different, isotopically distinct sources. For example, storms that arrived at 12am August 8th 2006 came from mainly westerly and southerly directions and generated precipitation with a δD of -44.3 ‰ and $\delta^{18}O$ of -5.5 ‰ respectively (Fig. 4.4a). At 12am on August 16th 2006, the storms were mostly northwesterly or westerly, which yielded a δD of -98.6 ‰ and a $\delta^{18}O$ of 12.1 ‰ (Fig.

4.4b). During November 30th 2006, southerly flow brought precipitation with a δD of -70.4 ‰ and a $\delta^{18}O$ of -7.3 ‰ (Fig. 4.4c). When the precipitation of December 19th 2006 was primarily derived from northwesterly and north directions, precipitation was more isotopically depleted with a δD of -134.6 ‰ and a $\delta^{18}O$ of -18.1 ‰ (Fig. 4.4d).

Age model and stratigraphy of the sediment core taken from Idavain Lake

Eleven AMS radiocarbon dates were obtained and calibrated to calendar years before 1950 (cal yr BP) (Table 4.1) using the IntCal04 calibration curve (Reimer et al., 2004) and CALIB 5.10 Beta program (Stuiver and Reimer, 1993). The median probability age output from CALIB 5.10 Beta was used as the single best estimate of the central tendency of calibrated age. A second-order polynomial curve provides the best fit for the radiocarbon dates and the two tephra dates (R^2=0.99) and is used as the age model (Fig. 4.5) throughout this paper. The extrapolated basal age for the core is 17,000 cal yr BP.

The Idavain Lake sediments are observed to be predominantly composed of organic silt, generally alternating between grey to black and brown to dark-brown gyttja and clay-silt layers (Fig. 4.6). The top 1260 cm consist of moderately organic-rich silt and numerous layers of tephra with variable texture and thickness. The bottom of the core is primarily composed of low organic grayish silt.

The MS values decrease towards the surface of the core, which is consistent with the core stratigraphy. The MS values are generally higher below ~1344 cm (15,000 cal yr BP), up to > 50 SI units/g (Fig. 4.6). The MS in this portion of core also fluctuates greatly

compared to other parts of the core. The MS ranges 45 and 33 SI units/g from ~1344 cm to ~972 cm (~12,500 cal yr BP) with a decreasing trend. From ~900 cm (12,500 cal yr BP) to the top of the core, the MS is relatively stable (< 30 SI units/g), with the exception of several visible tephra layers. There are abrupt increases in MS at several horizons such as 1359 cm, 1090 cm and 600 cm, corresponding to visible bands of tephra (Fig. 4.6).

LOI 550 °C values decrease towards the base of the core in general. Below 1344 cm, LOI is low, generally < 4 % at the core base. The LOI 550 °C values are relatively stable from 1344 cm to the surface of the core and slightly increase from1000 to 600 cm (12,000 to 8,500 cal yr BP) (Fig. 4.6). LOI 850 °C fluctuated between 0-4 % throughout the core, indicating that a negligible amount of carbonate is preserved in the sediments (Fig. 4.6).

C/N, $\delta^{15}N$ and $\delta^{13}C$ analysis of bulk sediments

C/N ratios of the Idavain core are relatively constant, ranging from 7 to 9 throughout the entire core (Fig. 4.6), with the exception of the top 2 cm and also between 16 to 20 cm, where the C/N ratios increase to 10 (Fig. 4.6). The $\delta^{15}N$ values from the Idavain core fluctuate dramatically but the values are low (1-4 ‰) (Fig. 4.6). The $\delta^{13}C$ values shift much more gradually compared to $\delta^{15}N$. From 1300 cm (15,000 cal yr BP) to the core surface, the overall $\delta^{13}C$ (Fig. 4.6) increases gradually. There are three abrupt increases from 1050 to 950 cm (13,000 to 12,500 cal yr BP), 750 to 600 cm (10,500 to 8,500 cal yr BP), and from 280 to 200 cm (4,000 to 3,000 cal yr BP).

δD and $\delta^{18}O$ analysis of chironomids

The range of variation in δD of chironomid head capsules ($\delta D_{chironomid}$) is -148.6 to -132.2 ‰, with a mean of -140.8 ‰ (Fig. 4.6). The precision determined from running samples in triplicate yielded a mean one standard deviation of 3.2 ‰ for $\delta D_{chironomid}$, which is identical to the precision from analyses of our internal standard.

The magnitude of variation in $\delta^{18}O_{chironomid}$ values throughout the Idavain core is large, ranging from 13.6 to 32.6 ‰, with a mean of 16.1 ‰ (Fig. 4.6). From 1400 cm to 1000 cm (16,000 and 12,500 cal yr BP), the $\delta^{18}O_{chironomid}$ increases gradually from 13.6 to 27.3 ‰ whereas the $\delta^{18}O_{chironomid}$ fluctuates dramatically above 1000 cm (after 12,500 cal yr BP). The magnitude of variations during the Holocene is up to 10 ‰. The precision determined from running samples in triplicate yielded a mean one standard deviation of $\leq$1.5 ‰ for $\delta^{18}O_{chironomid}$, which is a slightly lower precision that that from analyses of the internal standard (1.3 ‰). The magnitude of variation in $\delta^{18}O_{chironomid}$ far exceeds the sample precision (shown as the error bars in Fig. 4.6) and the highest $\delta^{18}O_{chironomid}$ (32.6 ‰) is at ~743.5 cm (10,300 cal yr BP). The chironomid abundance (head capsule counts/cm^3) varies throughout the core (Fig. 4.6). Overall, the abundance increases from the base of the core to its top and chironomid head capsules are most abundant at 400 cm.

Discussion

The modern Idavain Lake reflects primarily precipitation and it is not heavily influenced by evaporation. The δD and $\delta^{18}O$ of modern lake water at Idavain Lake are almost identical to the δD and $\delta^{18}O$ of the volume weighted mean peak precipitation season (July to October) (δD = -92.1 ‰ and $\delta^{18}O$ =-11.6 ‰), suggesting that the δD and $\delta^{18}O$ of lake water from Idavain Lake is strongly influenced by the δD and $\delta^{18}O$ of the peak precipitation season.

The δD and $\delta^{18}O$ of modern precipitation at King Salmon, which we use as a proxy for δD and $\delta^{18}O$ of precipitation at Idavain Lake, show a large magnitude of variation from August 2006 to July 2007 (Fig. 4.3a). This variation could be influenced by several factors including temperature, amount of precipitation, and different sources of moisture. The monthly mean temperature and precipitation amount do not correlate well with the δD and $\delta^{18}O$ of the precipitation, suggesting that differing sources of moisture are important in driving the δD and $\delta^{18}O$ of precipitation. The storm trajectories to King Salmon demonstrate that storms can originate from different moisture sources likely resulting in different isotopic compositions of precipitation, thus supporting this interpretation (Fig. 4.4). With a residence time of 3.3 years, Idavain Lake water likely provides an average reflection of the seasonal variability in precipitation and storm tracks.

LOI and MS data from the Idavain Lake core suggest that the nature of the sediments has changed over the last 16,000 years (Fig. 4.6), with increasing organic content toward the surface of the core. The negligible (LOI 850 °C < 3 %) amount of carbonate in

the sediment core indicates that the $\delta^{13}C$ data from the core reflects the isotopic composition of organic carbon. Low C/N ratios (< 10) (Fig. 4.6), with the exception of the top of the core, indicate that the organic matter preserved in the core is primarily derived from an aquatic source because plankton and algae C/N ratios are typically between 4 and 10 whereas terrestrial organic matter is greater than 10 (Meyers, 1997; Meyers and Ishiwatari, 1993). This implies that the $\delta^{13}C$ derived from the analyses of total organic carbon can largely be interpreted in terms of autochthonous primary production, with higher $\delta^{13}C$ values likely indicating higher primary production in the lakes as a result of phytoplankton discriminating less against $^{13}CO_2$ (Meyers, 1997; Meyers and Ishiwatari, 1993). Shifts towards higher autochthonous primary production occurred at 10,300 to 8,500 cal yr BP, 4,000 to 2,700 cal yr BP, and at ca. 1,000 cal yr BP.

Around ~1900 AD, the C/N is greater than 10. The absence of terrestrial macrofossils and reduced $\delta^{13}C$ at this time suggests the increased C/N could be due to the reduced proportion of autochthonous productivity, rather than increased terrestrial input. The timing of this change suggests it may be related to the Katmai eruption. One possible mechanism is that the ash from the eruption could have reduced the sunlight for a sufficient time to reduce productivity.

Changes in $\delta^{18}O_{chironomid}$ relate to changes in $\delta^{18}O_{lakewater}$ over the 16,000 years because the majority of oxygen in chironomid larvae is derived from water (Wang et al., in preparation). The magnitude of $\delta^{18}O_{chironomid}$ variation is as high as up to 14 ‰ during deglaciation and 10 ‰ during Holocene. One explanation for the changes in $\delta^{18}O_{chironomid}$ from Idavain Lake is that the data represent changes in temperature, as a result of

temperature driven changes in the isotope composition of precipitation (Dansgaard, 1964). The global relationship between the stable isotope composition of precipitation and temperature is 0.65‰/°C for higher latitude (Rozanski et al., 1993) similar to the relationship (0.69‰/°C) between precipitation $\delta^{18}O$ and instrument weather data in Bethel (Hu and Shemesh, 2003). A positive linear relationship between MAT and the $\delta^{18}O_{chironomid}$ has also previously been shown for a series of lake sites (Wooller et al., 2004). However, the highest $\delta^{18}O_{chironomid}$ (32.6 ‰) from Idavain Lake during the Holocene cannot plausibly be interpreted as a change in temperature as this would imply MAT of 27 °C, based on previously established relationships (Wooller et al., 2004). Admittedly, increased insolation since the last deglaciation has warmed southwest Alaska (Brubaker et al., 2001; Hu et al., 1995; Hu and Shemesh, 2003); however, this could not plausibly produce a MAT of 27 °C.

An additional suite of possible alternative explanations are needed for the large variation in $\delta^{18}O_{chironomid}$ from the Idavain core relate to changes in the $\delta^{18}O$ of past lake water besides simply temperature. The $\delta^{18}O_{chitin}$ of invertebrates is strongly influenced by the environmental water in which the invertebrates live in (Motz, 2000; Schimmelmann and Deniro, 1986c; Schimmelmann et al., 1987; Wooller et al., 2004; Wang et al., in preparation). Analyses of bark-feeding beetles along a climatic transect in Canada have demonstrated that chitin $\delta^{18}O$ is ~37 ‰ more enriched compared to environmental water (Motz, 2000), which corroborates the finding of ~42 ‰ more enriched $\delta^{18}O_{chitin}$ of chironomids compared to their growth water in a laboratory controlled environment experiment (Wang, unpublished data). Motz (2000) established a correlation of

$\delta^{18}O_{chitin}$=1.14 $\delta^{18}O_{environmental\ water}$+28.9 between invertebrate chitin and environmental water. Similarly, Wooller et al., (2004) derived a linear regression ($\delta^{18}O_{chironomid}$=1.28 $\delta^{18}O_{lake\ water}$+32.9) based on chironomid head capsules (which are primarily composed of chitin) and lake water data from a series of lakes ranging from mid-latitude (Massachusetts) to high latitude (Baffin Island). Because the location of Idavain Lake falls in the latitudinal range of lakes presented by Wooller et al., (2004), we used this linear regression to convert the $\delta^{18}O_{chironomid}$ values to lake water $\delta^{18}O$ values. The past $\delta^{18}O_{lake\ water}$ in Idavain Lake appears to have ranged from -15.1 to -1 ‰, with a mean of -7.9 ‰ over the last 16,000 years (Fig. 4.7a). If we assume that the $\delta^{18}O_{chironomid}$ from Idavain Lake are an indicator of $\delta^{18}O_{lake\ water}$, we then need a reasonable explanation for the large variability in $\delta^{18}O$ of past lake water (up to 10 ‰ during the Holocene and up to 14 ‰ from 12,500 to 16,000 cal yr BP).

A possible explanation is that the $\delta^{18}O_{lake\ water}$ was strongly influenced by marked changes in the precipitation/evaporation (P/E) balance (Gat, 1981). There are a number of reasons why this explanation seems unlikely. First, Idavain Lake is currently a topographically open lake and the modern $\delta^{18}O_{lake\ water}$ lies on the LMWL (Fig. 4.2), which indicates that Idavain Lake is relatively insensitive to evaporation today. Idavain Lake also receives about 500 mm/yr of precipitation and evaporation is less than 500 mm/yr in this area (Newman and Branton, 1972). Moveover, the residence time (3.3 years) for Idavain Lake also shows that evaporative alteration of lake water is unlikely (Sauer et al., 2001). Admittedly, extreme aridity events could have occurred in the past and caused lower lake levels through evaporation, which could subsequently have

resulted in an increase in $\delta^{18}O_{lake\ water}$. Based on the modern $\delta^{18}O_{lake\ water}$ (-11.1‰) from Idavain Lake, the MAT (0 °C) of the area (NCDC, 2004), the relative humidity (90%, NCDC, 2004) in the King Salmon area and the estimated lake volume (65 m^3), it would require an evaporative change in lake volume of 98 % to increase the $\delta^{18}O_{lake\ water}$ from the modern value (-11.1 ‰) up to the -1 ‰ value at 10,500 cal yr BP (based on the model presented by Curry et al., 1997). This does not seem reasonable as there is no indication of dramatic lake level decreases in the stratigraphy or MS data (Fig. 4.6) and no increase in the amount of carbonates (Fig. 4.6), which might have been expected if lake levels had altered dramatically. Similarly, increases in the $\delta^{18}O_{lake\ water}$ up to -8 ‰ at 4,200 cal yr BP would require a 16 % decrease in lake volume via evaporation. Such extreme evaporation would presumably concur with an arid event, resulting in vegetation to shift toward grass-dominated ecosystems, which are not evident in the pollen data from Idavain Lake (Brubaker et al., 2001).

A final alternative hypothesis to account for large variations in the $\delta^{18}O_{lake\ water}$ could be changes in the $\delta^{18}O$ of sources of moisture (e.g. precipitation). One scenario that could account for changes in the source of precipitation over time, and thus the $\delta^{18}O$ of precipitation, could be changes in the atmospheric circulation and thus moisture source. For instance, large variation in the $\delta^{18}O$ of precipitation from the individual rain events at King Salmon in August were observed, ranging between -20.2 to -1.5 ‰ (Fig. 4.1). The storm trajectories passing to King Salmon from August 2006 to July 2007 (Fig. 4.4) were derived from multiple moisture sources, which appear to relate to different isotopic compositions of precipitation. We propose that the $\delta^{18}O_{lake\ water}$ values in Idavain Lake likely reflect the ratio

of precipitation peak season to the rest of the year as well as also the relative proportion of major moisture sources. This approach is consistent with other studies that have used paleo $\delta^{18}O$ values to derive past changes in moisture sources during the late Quaternary (Amundson et al., 1996; Fisher et al., In press).

Two dominant atmospheric flow regimes were prescribed in a model derived by Fisher et al., (2004) and provide a mechanism to explain changes in the stable isotopic composition of precipitation for the GOA. Under a mixed (i.e. meridonal) flow regime, moisture sources include those from low latitudes (i.e. the tropic/subtropics Pacific) in addition to moistures from higher latitudes of the north and northwest Pacific, whereas a zonal flow regime primarily delivers moisture from higher latitudes of the north and northwest Pacific (Fisher et al., 2004). The mixed modern flow regime includes tropical water with ocean surface temperature up to 30 °C which advects warm moisture traveling along the northwest Pacific Ocean from a south and southeasterly direction. North Pacific zonal flow originates between 40 °N to 60° N and from the coast of Asia to North America (longitude 120 °E to 120° W) with ocean surface temperature around 10 °C (Fisher et al., 2004). The stable isotopic composition of the precipitation is isotopically less depleted because water vapor coming from the central North Pacific has rained out the heavier isotopes less (Dansgaard, 1964). The model also predicted the altitude effect between the two regimes. The difference between a mixed and zonal regime [($\delta^{18}O$ (zonal)- $\delta^{18}O$ (mixed)] are positive above an altitude of 1 km a.s.l. and negative below 1 km a.s.l. Therefore, the paleo $\delta^{18}O_{lake\ water}$ from Idavain Lake (Fig. 4.7) may reflect changes in the balance between the two main flow regimes associated with changes in atmospheric

circulation, modulated by radiative forcing and regional glacier advances (Bartlein et al., 1998; Edwards et al., 2001; Kutzbach et al., 1998). The $\delta^{18}O_{lake\ water}$ indices (Fig. 4.7) derived based on the mean $\delta^{18}O_{lake\ water}$ from Idavain Lake over the entire record and the anomalies relative to the means could therefore provide a record of changes in the balance of these two primary flow regime shifts since last deglacations.

Our reconstructed past change in atmospheric circulation patterns at Idavain Lake are consistent with other lines of evidence for environmental changes in the region. By 14,000 cal yr BP, the Cordilleran ice sheet had diminished and no longer had a strong influence, resulting in the typical westerly flow of air masses in Alaska (Kutzbach et al., 1998; Kutzbach et al., 1993). However, the pollen record from Idavain Lake (Brubaker et al., 2001) and Grandfather Lake (Hu et al., 2003) suggest that southwest Alaska experienced cooler, drier conditions than present, possibly indicating that if there were predominant zonal flows, it apparently did not affect the vegetation. The stable C/N ratio also demonstrates that the provenance of organic matter in the lake was also relatively stable since 16,000 years ago and was primarily aquatic in origin.

For the period from 14,000 to 12,800 cal yr BP GCM simulations show an increase in solar radiation (Kutzbach et al., 1998). *Betula* shrub tundra started to replace the mix herb tundra at this time at Idavain Lake and a gradual increase in primary productivity (indicated by an increase in the $\delta^{13}C$) also occurred. Paleoclimate records across the North Pacific from lake sediments (Anderson et al., 2005; Brubaker et al., 2001; Finney et al., 2004; Hu and Shemesh, 2003) and ice cores (Fisher et al., in press) indicate that south Alaska experienced significant climate variability since the Last

Glacial Maximum (LGM). After 12,800 cal yr BP, the $\delta^{18}O_{lake\ water}$ shows a marked positive anomaly (Fig. 4.7b), suggesting the dominant atmospheric flow shifted more to a southerly warm air mass.

Between 12,800 to 11,000 cal yr BP, the $\delta^{18}O$ data from the Idavain core indicate that the prevailing moisture sources in southwest Alaska was mixed modern flow. However, there was a slight decrease in primary productivity indicated by a decrease in $\delta^{15}N$ and $\delta^{13}C$ in Idavain Lake between 12,500 to 11,500 cal yr BP (Fig. 4.6). These changes coincide with a sharp reduction in *Betula* shrubs and an expansion in herb tundra communities in south central and south Alaska (Brubaker et al., 2001; Mann et al., 1998; Hu et al., 2003). A glacier re-advance at 12,800 cal yr BP was also documented along the GOA (Denton and Karlen, 1973). The slight decrease in $\delta^{18}O_{lake\ water}$ during this period also coincides with an advanced in glaciers during this period in the Katmai Mountain area (Pinney and Beget, 1991) and Ahklun mountains (Briner et al., 2002). The glacier advance also coincided with the decreased percentages of *Betula* pollen at several locations in southwest Alaska (Brubaker et al., 2001; Hu et al., 1995; Hu and Shemesh, 2003).

From 11,000 to 10,500 cal yr BP, the flow regime appears to have been dominanted by a zonal flow (Fig. 4.7). The isotopic anomalies are significant, indicating a marked shift in circulation. However, most other paleo-records are equivocal at this time. Pollen records contain increased grass pollen (sometimes indicating cooler/drier condition), but this occurs as shrubs and ferns are also increasing (indicating warmer/moister conditions) (Axford and Kaufman, 2004; Brubaker et al., 2001; Hu et al., 2002). The period also coincides with glacier advances on St. Elias mountain (Denton and Karlen, 1973). These results indicate

that the return to zonal atmospheric flow probably only had a minor effect on the vegetation, or that the effect was subtle and mediated by other factors, such as solar isolation, changing sea levels and extent of regional glaciations.

From 10,500 to 8,000 cal yr BP the $\delta^{18}O_{lake\ water}$ data suggest the dominant moisture source was a mixed modern flow regime with the exception of the period from 10,000 to 9,000 cal yr BP. Polypodiaceae pollen peaks at Idavain Lake after 9,000 cal yr BP (Brubaker et al., 2001), indicating increased moisture conditions. The inferred $\delta^{18}O_{lake\ water}$ value were the highest in the entire core by 10,300 cal yr BP., which also coincides with an expansion of *Populas* and *Salix* documented from ~10,700 cal yr BP in the Kenai lowland and suggesting warmer conditions in southwest Alaska at this time (Anderson et al., 2006). This was also concurrent with the increased percentage of *Populus* pollen and thermophilous beetles from the Arctic coastal Plain (10,800 cal yr BP) (Ager, 2003; Brubaker et al., 2001; Brubaker et al., 1983; Mann et al., 1998; Nelson and Carter, 1987) and expansion of *Populus* vegetation in the GOA (Mann et al., 1998), which suggest an increased summer temperature of 2-3° C.

At 8,000 and 6,000 cal yr BP the $\delta^{18}O_{lake\ water}$ in Idavain Lake were identical to the modern lake water values. During this period, other lakes in southwest Alaska are documented as having reached modern lake levels (Anderson et al., 2006). Glaciers re-advanced in the GOA (Calkin et al., 2001; Denton and Karlen, 1973) around 7,000 cal yr BP, which was accompanied by a predominant zonal flow regime (Fig. 4.7).

After 6,000 cal yr BP, GCM models indicate the simulated Pacific subtropical high was stronger than present, which resulted in a weakened Aleutian Low (Hewitt and

Mitchell, 1996; Lorenz et al., 1996) and allowed a predominant zonal flow to bring more westerly storms from the Northwest Pacific. Idavain Lake experienced a period of consistent zonal flow until 2,500 cal yr BP, with the exception of a period of weak mixed flow between 6,000 to 5,500 cal yr BP, when mountain glacier recessions were documented along the GOA (Denton and Karlen, 1973; Denton and Karlen, 1977). About 2,200 to 1,800 cal yr BP the $\delta^{18}O$ data from the Idavain core indicate a return to mixed flow, after which the flow was dominantly zonal until today. In general, pollen and glacial records indicate climatic cooling, increased storminess and renewed glacial activity in the GOA after 3,500 cal yr BP (Ager, 1983; Calkin et al., 2001; Heusser, 1983; Mann et al., 1998). The brief isotopic anomaly centered about 2,000 cal yr BP is not reflected in these records, although sampling sensitivity may explain this.

Conclusions

Viewed as a whole, Idavain Lake seems to have experienced atmospheric flow regime shifts during the Holocene. Some of the shifts are probably part of broad climatic trends that influenced vegetation changes in the region, where others are not. These flow regime shifts also correlate with coastal glacier expansions and contractions (Fig. 4.7) and the $\delta^{18}O_{\text{lake water}}$ in Idavain Lake may, therefore, provide a proxy record of shifts in moisture sources. The large variation in inferred $\delta^{18}O_{\text{lake water}}$ from Idavain Lake suggest southwest Alaska has experienced past changes in atmospheric circulation and climate dynamics over the past 16,000 cal yr BP. The zonal flow regime was seemingly dominant during the

periods from 16,000 to 13,800 cal yr BP, 11,000 to 10,500 cal yr BP, 10,000 to 8,000 cal yr BP, possibly a majority of the period from 8,200 to 3,500 cal yr BP, and also from 1,800 cal yr BP to present. A mixed modern flow regime was apparently dominant during the periods from 13,000 to 11,000 cal yr BP, 10,500 to 10,000 cal yr BP, 6,000 to 5,500 cal yr BP, and 2,300 to 1,800 cal yr BP. Our data suggest that changes in atmospheric circulation played an important role along the North Pacific Ocean.

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paper. This manuscript serves as partial fulfillment of the Doctorate in Philosophy degree at the University of Alaska Fairbanks for Yiming Wang.

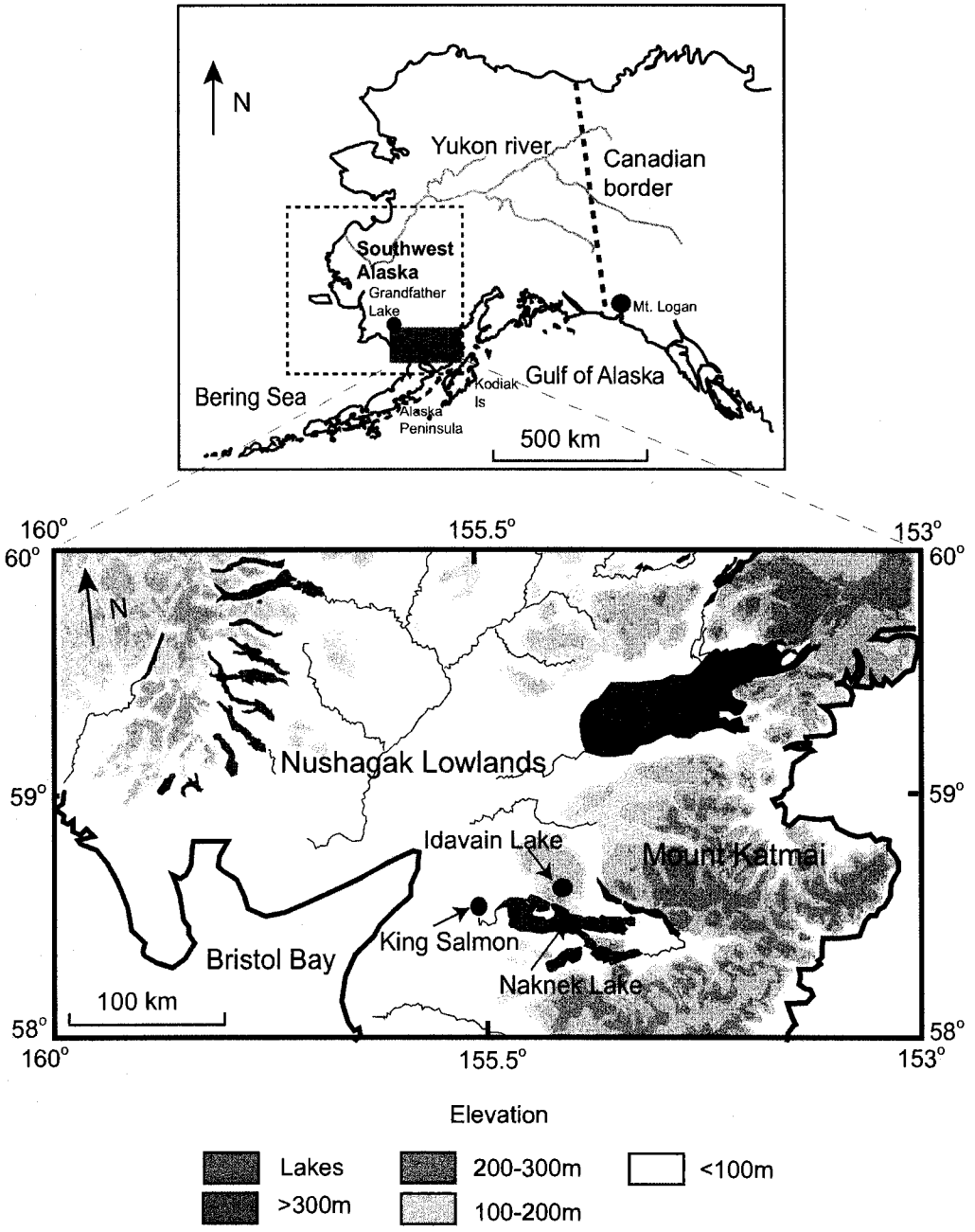


Fig. 4.1 The regional study area showing Idavain Lake and King Salmon weather station. Relavant sites mentioned in the paper are also shown.

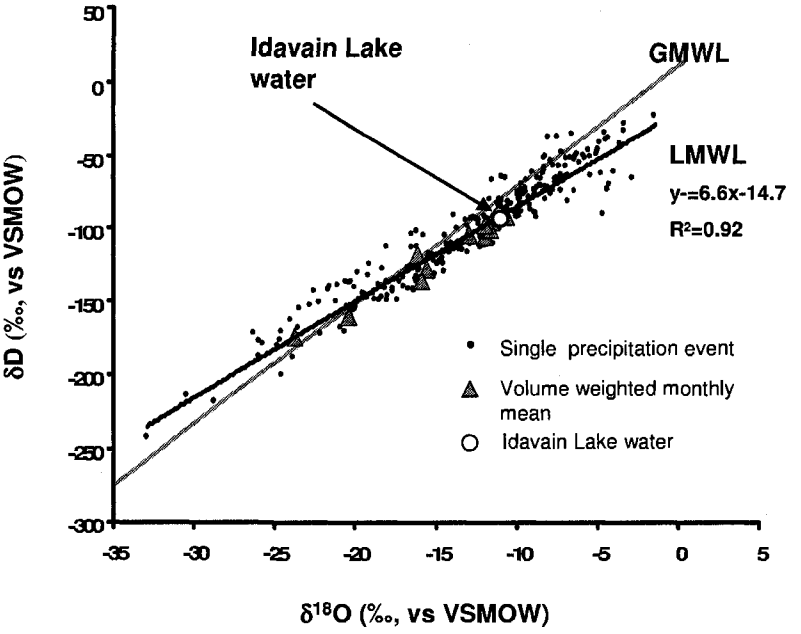


Fig. 4.2 The $\delta^{18}O$ and δD of precipitation from King Salmon. A Local Meteoric Water Line (LMWL) was plotted against the Global Meteoric Water Line (GMWL). Idavain Lake water data was also superimposed on the LMWL.

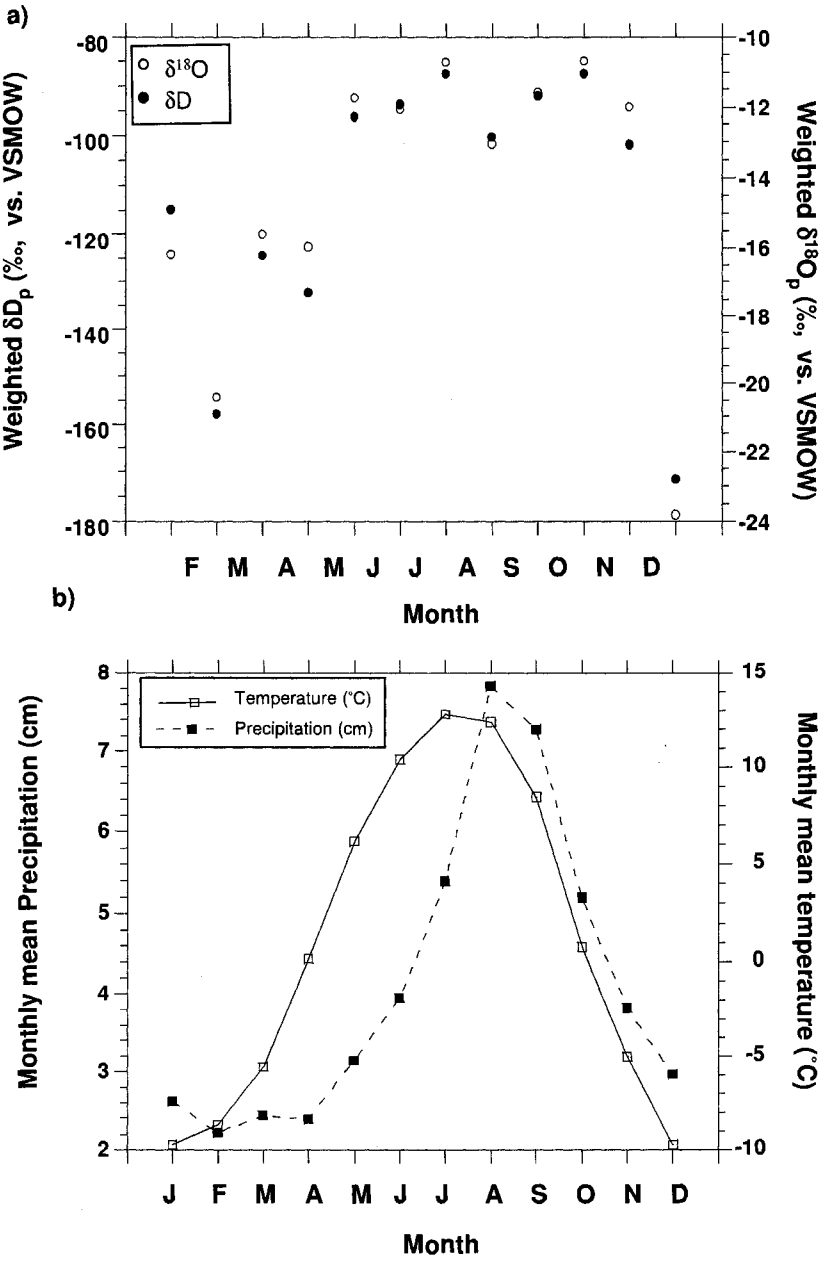
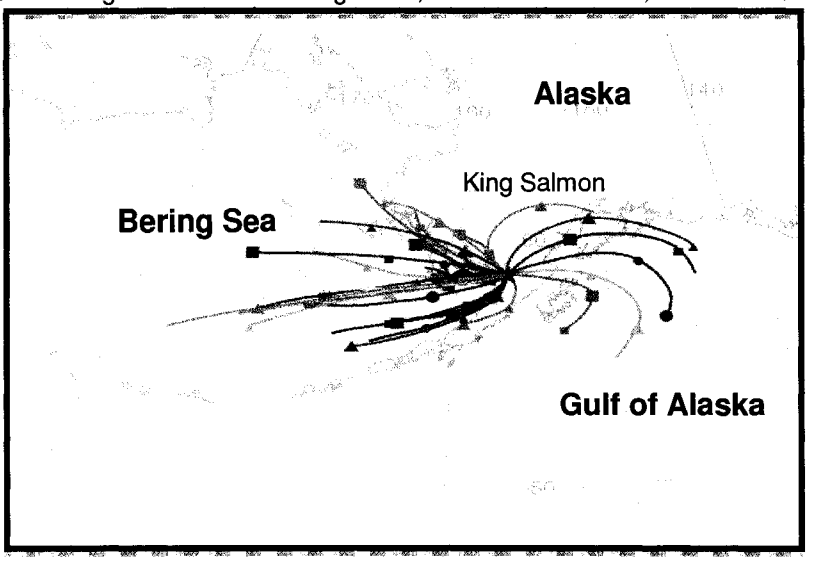


Fig. 4.3 a) The amount-weighted monthly mean of $\delta^{18}O$ and δD of modern precipitation collected at King Salmon weather station and b) mean precipitation amounts and mean monthly temperatures are based on a 62 year record from King Salmon weather station (NCDC, 2004).

a) 12 am August 2nd to 12 am August 8th, 2006: δD= -44.3 ‰, $\delta^{18}O$= -5.5 ‰



b) 12 am Aug. 10th to 12am Aug. 16th, 2006: δD= -98.6 ‰, $\delta^{18}O$= -12.1 ‰

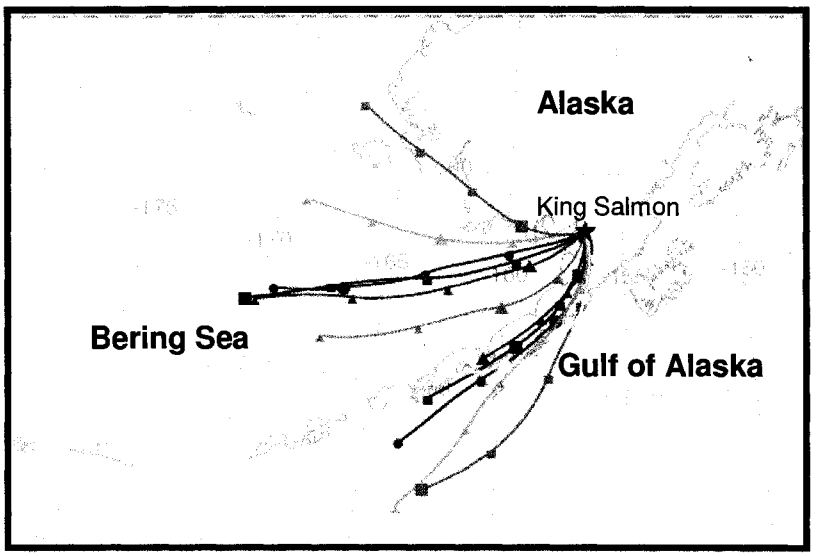
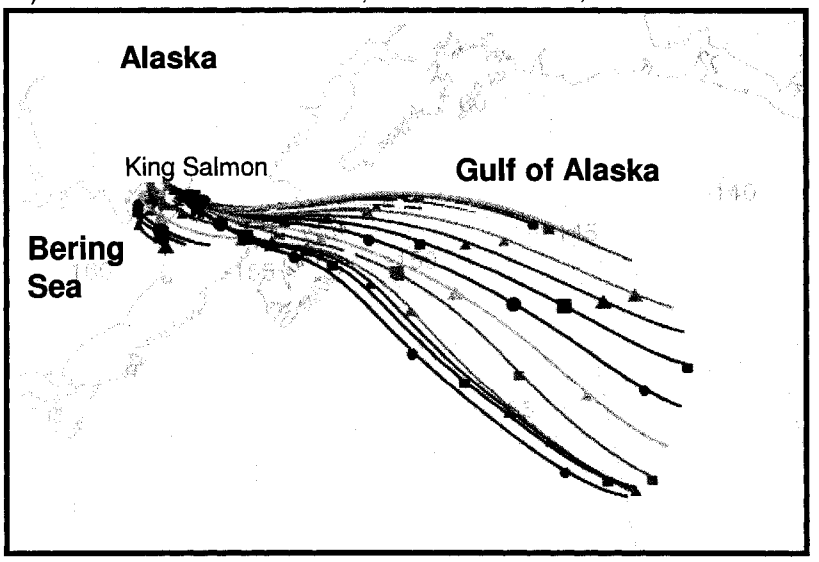


Fig. 4.4 (a-d) Examples of storm trajectories traveled ending at the King Salmon weather station over different periods and their isotopic compositions of precipitation. All the storm trajectories are tracked back a week prior to the ending time. Different colors present different storm directions and symbols along each trajectory indicate the position of the air parcel over the calculation period, the interval of which is defined by 24 hours here (storm trajectory sources derived from the Air Resources Laboratory (ARL)—http://www.arl.noaa.gov/ready.html.

c) Nov. 24th to 12am Nov. 30th, 2006: δD=-70.4 ‰, $\delta^{18}O$=-7.3 ‰



d) Dec. 13th to 12am on Dec. 19th, 2006: δD=-134.6 ‰, $\delta^{18}O$=-18.1 ‰

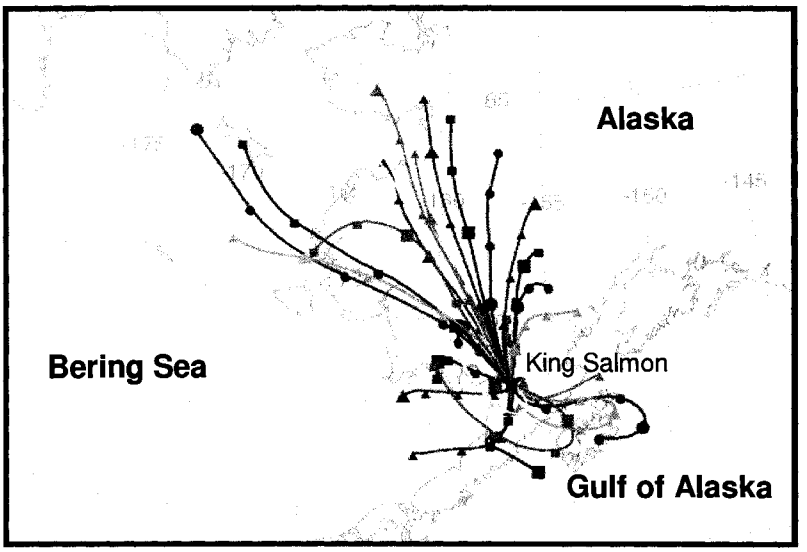


Fig. 4.4 (a-d) Continued

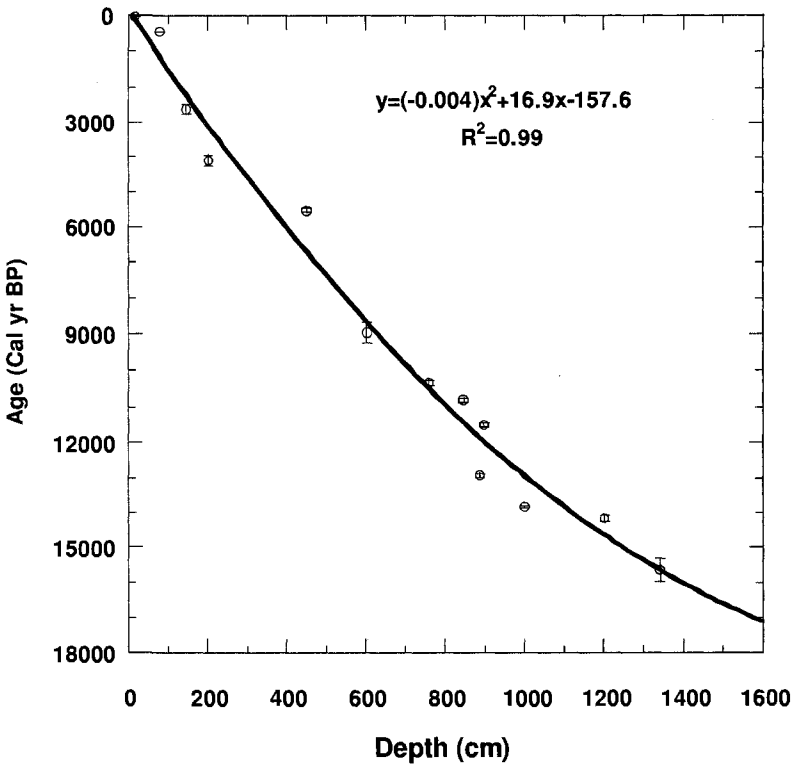


Fig. 4.5 Calibrated AMS radiocarbon age (with error bars) vs. depth curve for Idavain Lake core. The age model was based on a second order polynomial curve fit.

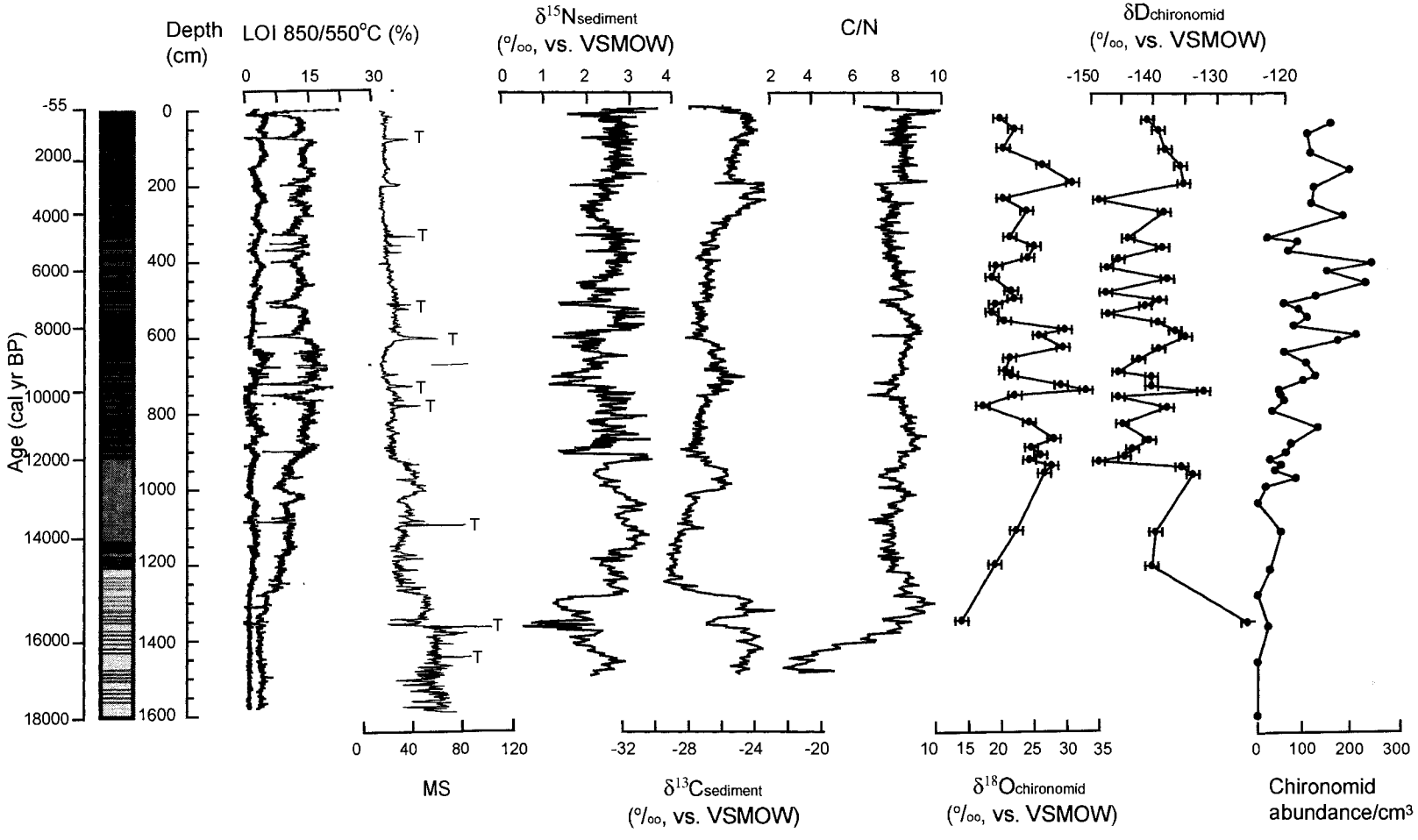


Fig. 4.6 Loss-on-ignition at 550°C and 850°C, magnetic susceptibility, $\delta^{15}N$, $\delta^{13}C$, C/N ratio of bulk sediments, $\delta^{18}O$ and δD of chironomid head capsules, and the abundance of chironomids from the Idavain core. T= Tephra

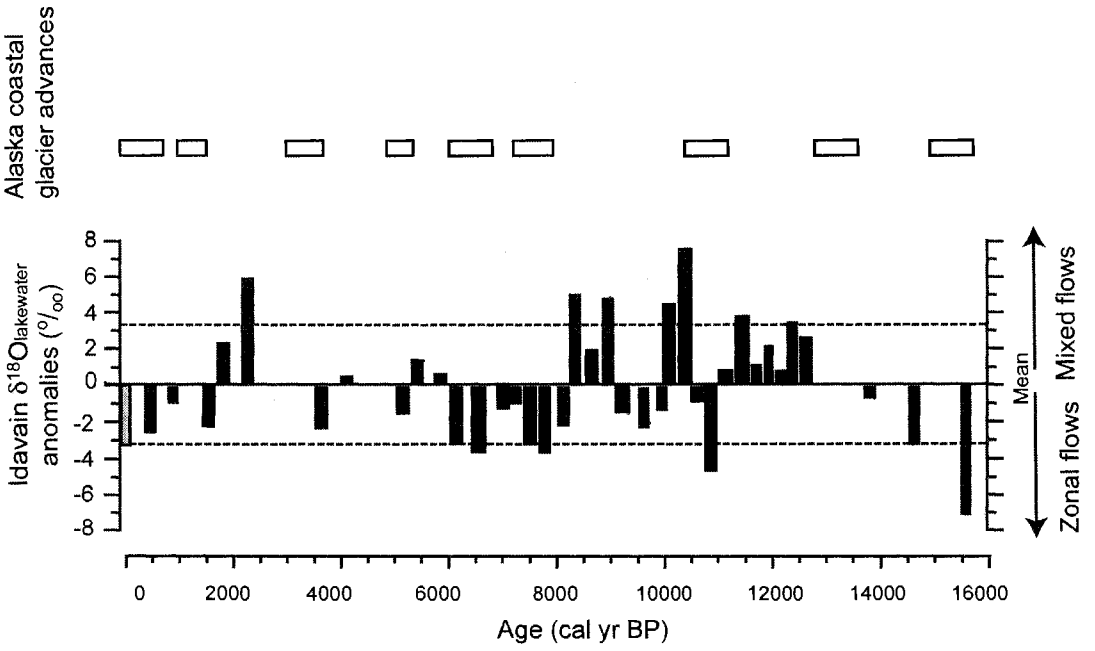


Fig. 4.7 The index of inferred $\delta^{18}O_{lakewater}$ anomalies since 16,000 cal yr BP and its comparison with glacier advances around the Gulf of Alaska since deglacation (Calkin et al., 2001; Denton and Karlen, 1973; Mann & Hamilton, 1995). Gray bar is the moden lake water value.

Table 4.1. List of tephra age, radiocarbon dates and calibrated age ranges for Idavain Lake, Southwest Alaska. The top of the core was estimated as 1950 AD.

Depth (cm)[a]	Material dated[b]	Laboratory number[c]	AMS ^{14}C age (yr. BP)	Calibrated mid-point of 1-σ age (cal yr. BP)[d]	1-σ range (cal yr. BP)[e]
0	Top of the core	n/a	0	-55	n/a
16	Tephra (Katmai ash)	n/a	93	38	n/a
78	Tephra*	n/a	380±35	415	327-503
146	Leaf and wood fragments	CAMS 132997	2560±60	2630	2503-2753
201	Leaf and wood fragments	CAMS 127190	3720 ±70	4078	3924-4232
451.5-452	Leaf and wood fragments	CAMS 124466	4800±35	5536	5481-5590
603-604	Leaf and wood fragments	CAMS 127191	8070±150	8950	8663-9237
760	Leaf and wood fragments	CAMS 124467	9180±45	10323	10252-10393
845.5	Leaf and wood fragments	CAMS 127192	9450±190	10793	10498-11087
888	Leaf and wood fragments	CAMS 124468	11005±35	12919	12888-12950
895-900	Leaf and wood fragments	CAMS 127193	9970±160	11494	11237-11750
1000	Leaf and wood fragments	CAMS 132998	11970±60	13827	13759-13895
1201.5	Leaf and wood fragments	CAMS 124469	12295±50	14152	14058-14246
1341-1344	Leaf and wood fragments	CAMS 127194	13230±220	15661	15325-15996

[a] Centered sample depth.
[b] Material dated are various proportions of terrestrial leaf and wood fragments
[c] All samples were dated at Lawrence Livermore National Laboratory
[d] Median probability CALIB v.5.10 (Stuiver and Reimer, 1993)
[e] 1σ age range
*78cm was correlated with the same tephra layer dated in a different core

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CHAPTER 5

General conclusion

Global environmental changes are of continued interest to scientists in all disciplines, and there is now a paramount need to gain a more comprehensive understanding of the impacts of climate change on the past to better predict possible changes in the future. A challenge of accurately interpreting the past stems partially from a lack of reliable proxies of past climate, especially at high latitudes. This thesis contributes to a further understanding and development of a new proxy using stable isotope analyses of subfossil midges (Chironomideae: Diptera) as an indicator of past environmental changes, particularly precipitation changes controlled by atmospheric circulation and other hydrological processes.

First of all I investigated and modeled the methodological considerations behind the analysis of small mass organic samples for their stable oxygen and hydrogen isotopic composition using continuous flow TC/EA isotope ratio mass spectrometry. I examined the blank effect associated with silver and tin capsules of various sizes and found that silver capsules (5x3.5 mm) from manufacturer A used in the study had the least blank effect. A mixing model demonstrated that the contamination associated with blanks can significantly influence results and that a blank correction is essential for small mass samples. At least a voltage of 0.5 (>0.05 mg, ~110 head capsules) is recommend for conducting stable isotope analyses on chironomid head capsule in order to obtain reliable data. This finding substantially reduces the number of fossil chironomid head capsules needed for stable isotope analyses compared to previous published research. A two step

transfer process is also recommended as standard protocol, which is less time-consuming and allows samples to be sorted and stored.

In order to better interpret the $\delta^{18}O$ and $\delta^{2}H$ variation of chironomid head capsules derived from lake sediment cores, I further examined the assumption that the stable oxygen and hydrogen isotope analyses of chironomids can be used as an indicator of lake water isotopes, and therein a proxy of precipitation isotopes. A controlled, replicated growth experiment quantified the degree to which the $\delta^{18}O$ and $\delta^{2}H$ of water and diet influence the $\delta^{18}O$ and $\delta^{2}H$ of chironomid larvae. About 70 % of the oxygen in chironomid larvae is proportionally derived from water and only about 30 % of hydrogen in chironomid larvae is derived from water. These findings are, interestingly, identical to the proportional contributions of oxygen and hydrogen from water and growth substrate supplied to microbial spores in a previously published study. These findings support that the stable isotopic composition of chironomids can be used to explain hydrological changes over geological time. Most notably, stable oxygen isotopes from chironomids better constrain habitat water isotopes, relative to hydrogen isotopes, since a greater proportion (~70 %) of chironomid oxygen is derived from water compared with the proportion of hydrogen derived from habitat water (~30 %). Conversely, hydrogen isotope analyses of chironomids better constrain dietary components relative to oxygen and would be a better marker for studying chironomid feeding ecology. The findings also add to the growing suite of comparative data available on the proportional contribution of oxygen and hydrogen deriving from water and diet available to organisms.

By applying stable isotope analyses on chironomid head capsules to a dated sediment core from southwest Alaska, I was able to reconstruct past climate and environmental change since deglaciation. Notably, the large $\delta^{18}O$ variation of chironomid head capsules taken from the sediments of Idavain Lake in southwest Alaska showed that the oxygen isotopic composition of precipitation varied up to ~10 ‰ during both the Holocene and deglaciation. The relatively stable $\delta^{2}H$ values of chironomids from the same core indicate that the isotopic composition of their past diet was relatively constant. Other lines of evidence drawn from C/N, $\delta^{15}N$ and $\delta^{13}C$ analyses of the same sediments showed that the productivity of the past Idavain Lake and organic matter origins were fairly stable over the same time period.

The large variation in $\delta^{18}O$ of chironomids from Idavain lake sediments may reflect alternating shifts in atmospheric flow regimes that brought precipitation with different stable isotope compositions to southwest Alaska. A zonal flow regime appears to have been dominant from 16,000 to 13,800 cal yr BP, 11,000 to 10,500 cal yr BP, 10,000 to 8,000 cal yr BP, and during a majority of the periods from 8,200 to 3,500 cal yr BP and from 2,000 cal yr BP to present. A mixed modern flow regime seems to have been dominant during the periods from 13,000 to 11,000 cal yr BP, 10,500 to 10,000 cal yr BP, 6,000 to 5,500 cal yr BP and 2,500 to 1,800 cal yr BP. These shifts in moisture regimes appear to coincide with a series of glacier advances and recessions along the Gulf of Alaska.

A future research direction is to examine the relationships between specific compounds (e.g. chitin) from chironomids to water and diet. The proportional

contribution of oxygen and hydrogen from diet and water to those of whole chironomid larvae, which includes amounts of lipid and protein, may differ from those of chitin, the predominant material preserved in the fossil record. Nevertheless, this PhD research has demonstrated that $\delta^{18}O$ and $\delta^{2}H$ of subfossil chironomid head capsules is a promising tool as an indicator of past environmental changes. Combined with multiple stable isotope analyses of sediment the $\delta^{18}O$ and $\delta^{2}H$ analyses of chironomids can provide a means for revealing past environmental changes, especially those related to changes in atmospheric circulation patterns.